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ALUMINIUM UPTAKE AND TOXICITY IN PLANTS

by L. H. JONES

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INTRODUCTION

The occurrence of active aluminium as a component of the soil acidity complex causing injury to plants has long been recognized (Hutchinson [7]). The application of lime, however, affords a simple cure for soil acidity problems and consequently interest in aluminium as a plant toxin has been almost lost. Magistad [10] showed that it could be absorbed by plants at pH values above 7.5, when it becomes soluble as the aluminate anion. In 1943 Allen [1] succeeded in producing blue Hydrangea flowers under alkaline conditions, and this was attributed to the uptake of aluminium as aluminate.

Recently the question of aluminium uptake at high pH values has been shown to be of more than academic interest by Rees and Sidrak [12 13], who were working on the problems of reclamation of pulverised coal ash (fly-ash) deposits produced by the Central Electricity Authority Power Station at Hams Hall in Warwickshire. They found that high levels of aluminium accumulated in plants growing on fly-ash at a field pH of between 8.5 and 9. This investigation has now been extended to include a study of the effects of ash from a number of different power stations distributed across England and Wales. The discovery that aluminium was playing a part in the nutrition of plants grown on fly-ash has led to the necessity of finding out more about the fundamental mechanism of its absorption and translocation, especially under alkaline conditions. This paper describes some preliminary experiments and discusses their possible significance.

MATERIAL AND METHODS

1. *Extraction of fly-ash for "available" aluminium*

Fly-ash samples were air dried and extracted with 10 volumes of acetic acid/sodium acetate buffer, pH 4.8 for 6 hours in an end-over-end shaker at 48 rpm (Rees and Sidrak [13]). The extracts were filtered and stored for use.

2. *Plant material*

Hordeum vulgare var. Herta seedlings were germinated in sand and transplanted after 1 week into 7-inch bitumenized pots containing either fly-ash or soil, 7 plants being placed in each pot. The plants were watered regularly with tap water, and harvested after five weeks. The leaves were rinsed twice in distilled water and dried to constant weight at 95°C.

Roots of *Pisum sativum* var. Meteor were obtained from 4-day-old dark-grown seedlings germinated in vermiculite.

Hordeum vulgare var. Sacaramento seedlings were germinated on racks over a constant-temperature waterbath at 25°C for 7 days. Seedlings of mangold (yellow globe) were harvested after 28 days germination in vermiculite.

Mustard plants, grown on ash–podsol mixtures of different pH values, were kindly supplied by Miss R. L. Weston.

3. *Preparation of root macerates*

Root material (20 g) was macerated with 100 ml of acetic acid/sodium acetate buffer at pH 4.8 in a Waring Blendor for 2 minutes. Coarse particles were removed by filtration through nylon gauze, fine sediment was removed by centrifugation for 5 minutes at 2400 × g.

4. *Chemical analysis*

Oven-dry plant material was wet-ashed using a mixture of nitric, perchloric, and sulphuric acids as described by Piper [11]. Aluminium was determined by the eriochrome cyanine method (Jones and Thurman [8]); pH measurements were made with the glass electrode, and phosphates were estimated by the amino-naphthol-sulphonic acid method of Wolf [18].

5. *Chromatography*

An investigation was made into the use of chromatographic techniques for the separation of aluminium–organic acid complexes using a number of solvent systems. Dissociation of the complexes in acid solvents led to the adoption of a basic solvent system, separation being obtained using methanol: ammonium carbonate (aq. saturated) 75 : 25 v/v. (Roberts *et al.*[15]). Aluminium was detected by its fluorescence under ultraviolet light after spraying with a solution of 8-hydroxyquinoline in alcohol. (Elbeih, Mc. Ombie and Pollard [5]). Unfortunately this reagent is not specific for aluminium (Reeves

and Crumpler [14]) but trials with calcium and magnesium, which were likely to produce the most interference, showed that in the solvent system used, only diffuse fluorescent streaks were produced by these elements, and the aluminium spots could still be discerned. The complexes were prepared from solutions of the free acids and aluminium sulphate, uncomplexed aluminium being precipitated by ammonia and filtered off. A summary of the separations obtained is shown in Table 1.

TABLE 1

R_f values of carboxylic acids and their aluminium complexes
Solvent: methanol: saturated ammonium carbonate (75 : 25 v/v).
Whatman No.-1 paper, descending 6 hours, temperature 25°C.

Acid	R_f acid	R_f complex	Remarks
Succinic	0.55	—	No complex
Malic	0.46	Streak to 0.30	Weak
		0.53	Weak
Tartaric	0.43	0.05 to 0.16	Weak
		0.43	Strong
Citric	0.28	0.24	Strong
Oxalic	Streak to 0.34	0.05 to 0.16	
		0.30	Strong
Phosphoric	0.17	—	No complex
Cations	R_f	Fluorescence with 8-hydroxyquinoline	
Sodium	0.52	None	
Potassium	0.45	None	
Calcium	Streak to 0.54	Faint fluorescence	
Magnesium	Streak to 0.75	Faint fluorescence	

The chromatograms were cut into one inch squares and assayed for aluminium by a semi-micro modification of the eriochrome cyanine method. Good correlation was obtained between the fluorescent areas and the distribution of aluminium.

EXPERIMENTS AND RESULTS

1. *Mobility of aluminium in alkaline media*

Wide differences in "available" aluminium were found in samples of fly-ash from 8 different power stations distributed across England and Wales, and analysis of barley (var. Herta) tops grown on these ashes showed some correlation between "available" aluminium and aluminium accumulated, (correlation coefficient 0.854), (see Table 2). Magistad [10] however, showed that aluminium uptake was governed by its solubility, which in turn is controlled by pH; so that the pH of the ash, which varied between 6.45 and 9.01, would be expected to be the major factor in determining aluminium

availability. Maclean and Gilbert [9] on the other hand have shown that barley roots are capable of absorbing aluminium from colloidal aluminium hydroxide in contact with them, and thus the potentially available aluminium will also play a part in supply of this element.

TABLE 2

Relationship between ash pH, "potentially available aluminium", soluble aluminium and the aluminium content ot barley tops

Power station	Ash pH	Potentially available Al (ppm)	Soluble Al (Magistad) calculated from pH	Al content barley tops ppm
1	8.20	710	6	112.5
2	8.38	625	12	107.5
3	8.88	148	30	70.0
4	8.51	220	25	52.5
5	7.98	168	4	37.5
6	8.29	52	10	37.5
7	9.01	24	(45) 24	27.5
8	6.45	230	0.2	10.0

The partial correlation of $[Al]_{plant}$ against $[Al]_{ash}$ corrected for pH is 0.976, which is highly significant at the $P = 0.1$ level. Since pH plays a part in aluminium availability in alkaline media it is evident that under the conditions obtaining in fly-ash, active aluminium is mobile as an aluminate ion. The chemistry of the aluminates is complex and the hydrated forms $Al(OH)_4^-$ and $Al(OH)_6^{\equiv}$ probably exist in solution. For simplicity, however, the older usage of AlO_2^- will be used to designate aluminate in this paper.

2. *Fixation of aluminium* in vitro *by plant root macerates*

Attempts to estimate aluminium directly in root macerates by the eriochrome cyanine method failed, and added aluminium could not be recovered. Spectrophotometric measurements indicated that the cause was fixation of aluminium rather than an attack on the dye molecule.

A comparison was made between the aluminium-fixing capacity of root macerates of three plants of varying resistance to aluminium toxicity, *viz* barley (sensitive), pea (semi-tolerant), and mangold (tolerant). It will be seen (Fig. 1) that there is a correlation between the capacity to immobilize aluminium and the physiological resistance to its toxic effect.

An attempt to characterize the aluminium-fixing agent pre-

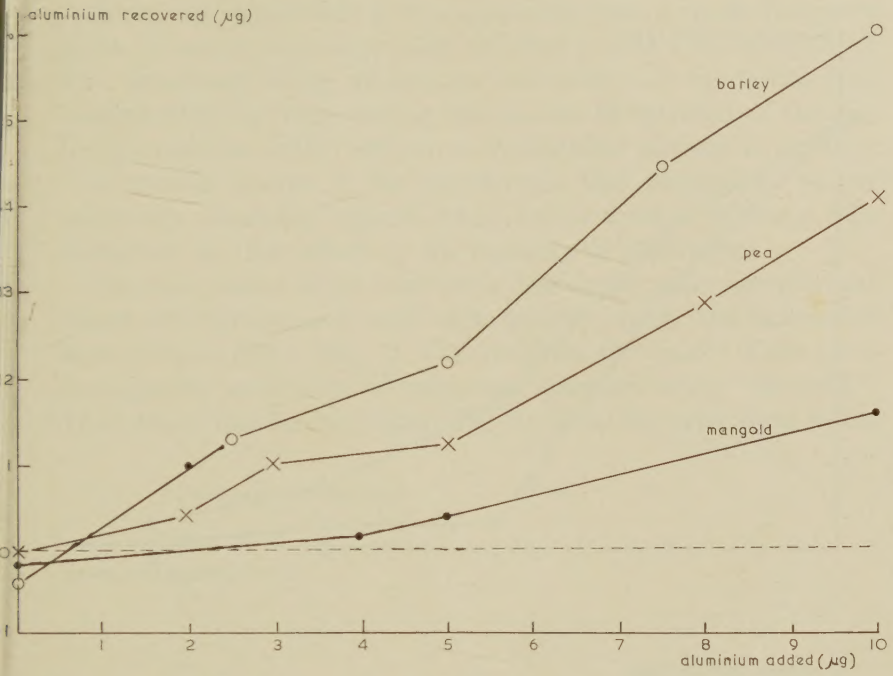
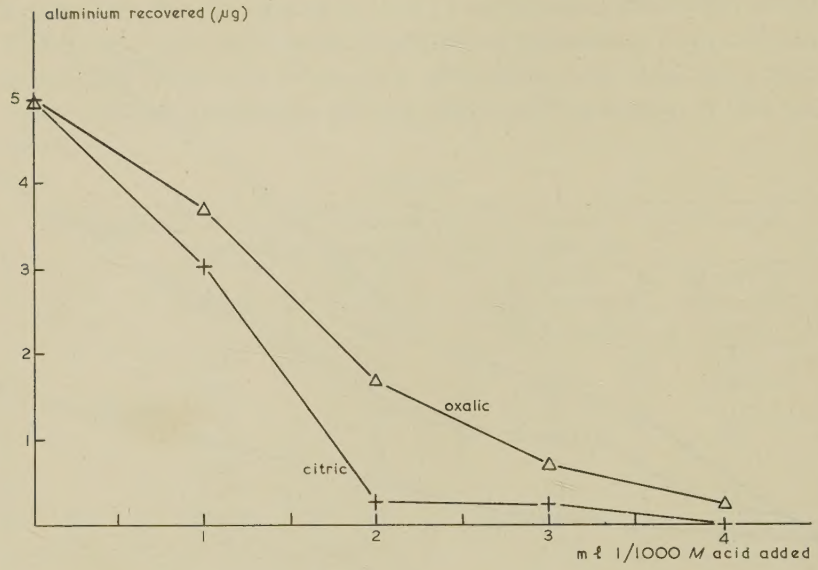


Fig. 1. Effect of addition of 1 ml of root macerate (0.2 g fresh root material) on recovery of added aluminium.

venting the formation of aluminium–eriochrome cyanine complex was made as shown in Table 3, dialysis being carried out directly on unfiltered macerates.

TABLE 3

Characterisation of agent preventing formation of aluminium–eriochrome complex		
Test	Result	Inference
Wet ash sample with HNO_3, $HClO_3$, and H_2SO_4	Interfering activity lost	Probably organic
Dialysis	Activity lost from dialysate, recovered from outside membrane	Small, non protein molecule
Boil for 1 hour	No loss of activity	Thermostable
Pass through Amberlite I R 120 H^+	Activity retained in effluent	Not cationic or amino acid
Pass through Amberlite I RA 400 OH^-	Activity lost from effluent. Recovered on elution with 4% NaOH	Anionic



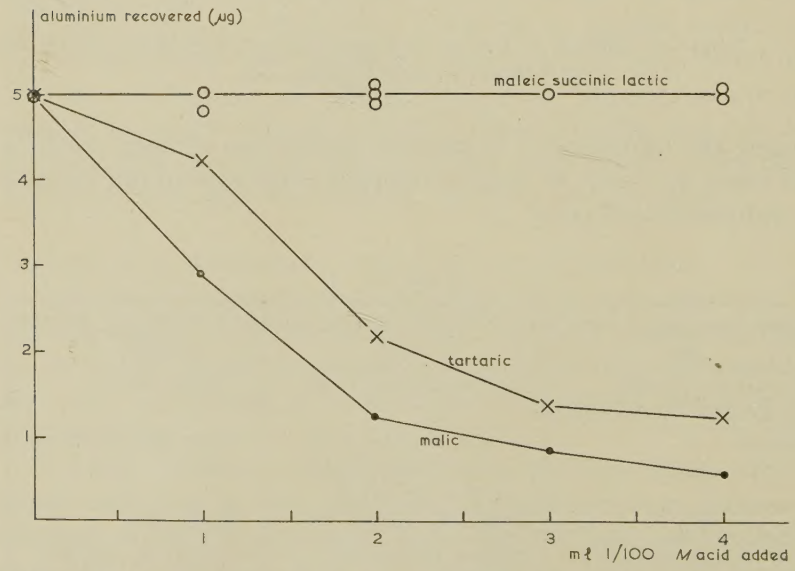


Fig. 2. Effect of addition of organic acids on recovery of aluminium. (5 μg Al in all samples).

From this experiment it was concluded that a small dialysable anion (or anions) was responsible and that protein was not involved. The aluminium-fixing substances were extracted by dialysis and concentrated by freeze-drying (or vacuum distillation) of the dialysable fraction, which effected a considerable increase in activity.

A possible source of the interference was thought to be the commonly occurring organic acids, and a number of these were examined for their effect on the recovery of aluminium.

The most active acids were citric and oxalic, some activity was shown with tartaric and malic while succinic, maleic and lactic acids were without effect (Fig. 2). This confirms the results of the chromatographic separation of complexes described under "Methods". The extract interference curve (Fig. 3) shows the same form as the

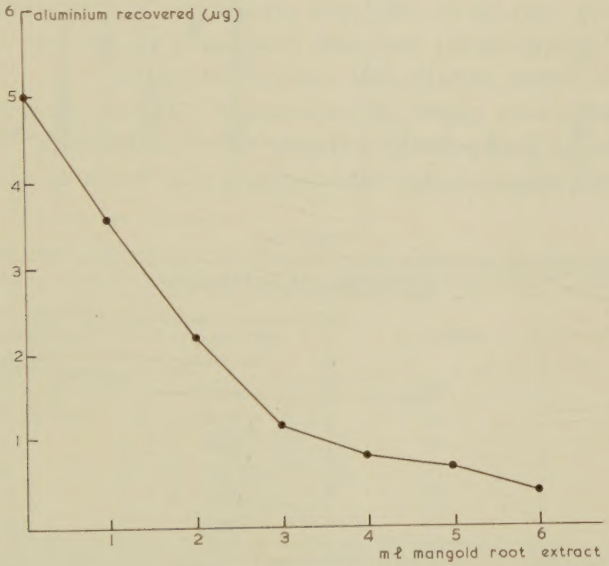


Fig. 3. Effect of addition of concentrated mangold root extract (ml, arbitrary units) on recovery of aluminium. (5μg Al in all samples).

malic acid curve, and this acid would be expected to be present in the greatest quantity.

In vitro tests showed that in the presence of these acids neither aluminium hydroxide nor phosphate can be precipitated by NaOH at any pH values.

3. *Chromatographic separation of aluminium complexes in plant material*

The elucidation of the mechanism of aluminium absorption from an alkaline medium and the subsequent transport of this element in plants poses a number of problems. The discovery that aluminium-complexing substances were present in roots suggested that aluminium might be transported in the complexed form, and led to the idea of attempting to isolate such complexes from plants grown on an aluminium-rich medium. In order to facilitate detection of any possible aluminium compounds, a plant insensitive to aluminium toxicity and known to have a high rate of aluminium uptake was required. Mustard was selected for this purpose, as greenhouse experiments showed that when supplied with aluminium there was little toxic effect, the phosphate status of the tops was not affected, and up to 350 ppm aluminium accumulated in the leaves. The experiment was also designated to test the effect of pH on aluminium uptake and complex formation.

The plants were grown on mixtures of different proportions of fly-ash and an acid podsolic soil also high in aluminium. The mixtures gave a range of pH values as shown in Table 4, which also

TABLE 4

Relationship between ash–podsol ratio, pH, and theoretically soluble aluminium

Ash, %	Podsol, %	pH mixture	Soluble Al (ppm) calculated from Magistad
1	99	4.55	4
2	98	4.63	3
5	95	5.33	1
25	75	7.78	4
50	50	8.13	6
75	25	8.48	16
100	0	8.75	25

shows the theoretical levels of soluble aluminium. The plants were harvested after 38 days growth and the roots were washed thoroughly in a jet of water. They were blotted dry, rolled carefully into small balls and squashed between two sheets of glass on to Whatman No.–1 chromatographic paper. (Greenshields [6]). The exudate was dried rapidly in a current of warm air and the chromatogram eluted with the methanol/ammonium carbonate solvent as de-

scribed previously. A tracing of the fluorescence visible under ultraviolet light after spraying with 8-hydroxyquinoline is reproduced in Fig. 4, together with the separation obtained with known standards.

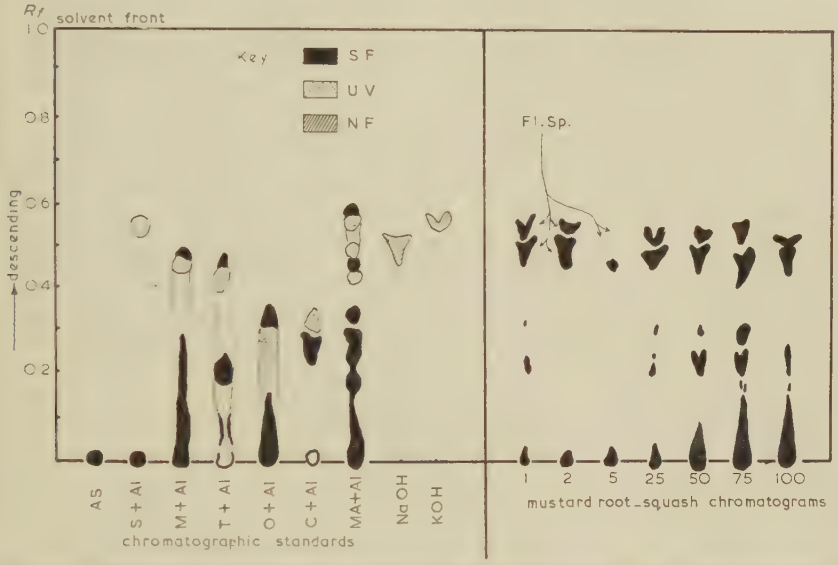


Fig. 4. Diagram of fluorescence obtained after spraying the chromatograms with 8-hydroxyquinoline.

Solvent: Methanol/saturated ammonium carbonate 75/25 v/v.
Explanation of abbrevations: –

Key: – SF – strongly fluorescent = aluminium
UV – U.V.-absorbing = acids
NF – non fluorescent, blue with bromphenol blue = cations

Chromatographic standards: – AS — aluminium sulphate
S + Al — succinic acid + Al
M + Al — malic acid + Al
T + Al — tartaric acid + Al
O + Al — oxalic acid + Al
C + Al — citric acid + Al
MA + Al — mixed acids + Al

Mustard root-squash chromatograms: – 1, 2, 5, 25, 50, 75, and 100 – percent ash in ash—podsol mixture with pH values of 4.55, 4.63, 5.33, 7.78, 8.13, 8.48, and 8.75 respectively. *In chromatogram:* – Fl. Sp. – fluorescent spots containing Na (R_f 0.48) and K (R_f 0.53).

Fluorescence occurred corresponding with the R_f values of citric, oxalic and malic acid complexes, and ninhydrin spray showed that amino acids were absent from these areas, other complexing substances may of course have been present and no positive conclusions can be drawn regarding the identity of the complexes. It should be noted that the distribution and intensity of the fluorescence in the different samples corresponds with the solubility of aluminium

in the growing medium. The two large spots at R_f 0.48 to 0.50 and 0.53 to 0.54 corresponded exactly with basic spots containing sodium and potassium ions respectively (shown by elution and flame photometry), which however, do not themselves form fluorescent 8-hydroxyquinolates. If the fluorescence was due to aluminium, it seems likely that the spots represent sodium and potassium aluminates formed from alumina or aluminium hydroxide in the presence of the alkaline solvent system.

A similar experiment with barley roots produced strong spots at R_f 0.48 and 0.53, with some streaking away from the origin, but no sign of a "citrate" spot.

4. *Relationship between the aluminium and phosphate content of the leaves of plants grown on soil and ash*

Leaves of plants grown on Hams Hall ash in pots in the greenhouse were analysed for aluminium and phosphate, and the results compared with similar analyses of plants grown in soil. The data shown in Table 5, which are compounded from the results obtained

TABLE 5

Phosphate and aluminium status of plants grown on soil and ash

Plant	Phosphate %		Aluminium ppm		Al tolerance
	Soil	Ash	Soil	Ash	
Barley	2.30	0.97	22	109	Sensitive
Brussels sprouts	1.58	0.95	13	51	Semi-tolerant
White clover S. 100	1.02	0.90	54	310	Tolerant
Mustard	1.82	1.29	14	350	Tolerant
Atriplex hastata var. *deltoidea*	1.55	1.21	101	335	Tolerant

"Available" levels of elements in ash PO_4 1125 ppm; Al 710 ppm (Morgans extract, 1 hour, end-over-end shaker). pH 8.2. Soil: Al 34 ppm, pH 4-8

from several different experiments, show that although barley and sprouts are unable to maintain their phosphate status on ash, clover, mustard, and Atriplex are much less affected, in spite of a much higher intake of aluminium. The severity of toxicity symptoms observed is recorded in the last column of Table 5, where the plants are rated for aluminium tolerance.

DISCUSSION

Experiments have shown that mobile aluminium is present in fly-ash at high pH values, presumably as a soluble aluminate, and

that it is available to plants grown in the ash. Since aluminates are hydrolysed and the hydroxide is precipitated at pH levels below 7 it seems unlikely that aluminium could enter the plant as the anion, as the root surface has been shown by many workers using different species to be consistently on the acid side of neutrality (Small [16]). Indicator studies on clover roots *in situ* in fly-ash showed that the root surface was below pH 6 in a medium of pH 8.5. Thus around the roots of a plant growing in an alkaline medium there must exist a narrow zone where the pH changes rapidly. The width of this zone will depend on the influence exerted by the root on the rhizosphere, the nature of root exudates, and the extent and activity of the microflora. In the absence of complexing substances the mobile aluminium diffusing into this zone would be expected to precipitate either as hydroxide or phosphate. In order for aluminium to be absorbed by the plant it must therefore either be mobilised from the precipitated form, (Maclean and Gilbert [9]) or prevented from precipitating in the pH transitional zone by the production of complex-forming compounds. In the absence of such a mechanism a physical block to the diffusion of other nutrients might well be formed both on the root surface and in the free space, especially if the roots were not exerting much influence on the surrounding alkaline medium. This would approach the conditions found by Wright [19 20] and Wright and Donaghue [21] using barley. One of the major symptoms of aluminium toxicity is that of induced phosphate deficiency in the tops, accompanied by an accumulation of both aluminium and phosphate in the roots, and this would suggest such a physical blockage by precipitation (Table 5). Many plants, however, are not affected by high levels of aluminium, at least under acid conditions, and they are able to maintain their phosphate status although in many of these plants aluminium is accumulated rather than excluded (Chenery [3]). For aluminium to be transported it must remain in a soluble form at physiological pH values in the presence of phosphate. Again this suggests the presence of complexes. In his excellent review articles Hutchinson [7] states that "Aluminium forms complex ions with certain organic acid radicals, but the most obvious of these, the aluminoxalate and aluminicitrate ions have not so far been reported". He also remarks that "a far more extended qualitative and physiological study would be of great interest,

particularly in relation to the pH and nature of the organic acids of sap". The experiments on the complexing capacity of root macerates suggest that complexing agents are present in plants and there is some indication that the agents concerned may be the commonly occurring organic acids malic, citric, and possibly tartaric and oxalic. This is supported by the chromatographic evidence which shows that complexes were formed in mustard roots, and that they possessed R_f values corresponding with those of the complexes of aluminium with the organic acids mentioned.

Since high levels of soluble aluminium usually occur in nature only under acid soil conditions, it is to be expected that acidiphilous plants have evolved resistance to aluminium toxicity. On the other hand, alkaliphiles, alka-tolerant, and many mesophilous plants do not normally come into contact with this ion, and any resistance they may possess is purely coincidental. Chenery [3] examined a large number of plants and found that all aluminium accumulators studied had a cell sap pH of less than 5.3.

Small [16] has shown that acidiphilous plants generally possess strong organic acid-buffer systems in the cell sap in contrast to alkaliphiles where phosphate is the predominant system. Alka-tolerant plants, on the other hand may be of two types. Firstly those with a strong phosphate-buffer system dominating a weak organic acid system; and secondly those with a high concentration of organic acids not likely to be neutralized by the external medium.

It is among the latter group that aluminium tolerance at high pH values would be expected if the presence of excess organic acids is operative in preventing aluminium precipitation at the root surface and in maintaining its mobility. In fact this requires the exudation of acid into the rhizosphere, and the assumption is made that plants containing large quantities of organic acids in their cell sap are more likely to release traces of these substances. Active accumulation of the chelated aluminium will be governed by the plant's system of solute uptake and the speed of penetration of uncharged molecules, presumably into the apparent free space by diffusion (Briggs and Robertson [2]). DeKock and Mitchell [4] showed that chelated aluminium in the form of the ATA, DTPA EDTA complexes was absorbed from nutrient solutions by mustard plants. They suggested that the residual charge on the chelate molecule was an important factor governing uptake, molecules

with no charge or a single charge being more easily absorbed than those with two or more charges. This would favour uptake of trivalent metals such as iron and aluminium, and these considerations may well hold good for the uptake of aluminium-organic acid complexes.

Of the plants examined, barley is known to be an alka-tolerant plant of the phosphate-buffer type, while *Beta maritima*, closely allied to *Atriplex hastata*, which is one of the primary colonizers of fly-ash deposits, is of the second, high-acid variety (Small [16]). Clover, sprouts, mangold, and mustard are all alka-tolerant but as yet their buffer systems have not been examined. It is of interest to note, however, that Wallihan [17] using ladino clover, was unable to find the accumulation of aluminium and phosphate in the roots reported by Wright [19 20] who worked with barley. The controversy between these workers would be resolved if it could be shown that the two possessed different cell-sap buffer systems, or exuded different amounts of organic acids regulating aluminium solubility. Working with S. 100 white clover grown on ash (Table 5) the author has found little evidence of PO_4 deficiency and a considerable accumulation of Al in the leaves in contrast to the condition in barley which is very sensitive to excess aluminium.

It should be pointed out that although this approach to the problem of aluminium uptake was inspired by the problems associated with alkaline media, the proposed mechanism would apply equally to plants growing under acid conditions, since physiological pH values are normally such as to preclude the existence of free Al ions. This work may also have a bearing on the problem of the uptake of iron and other heavy metals.

SUMMARY

Mobile aluminium has been found to be present in fly-ash deposits at high pH values. The problem of how plants can absorb aluminium from the anionic form is discussed, and it is suggested that organic acids produced by the plants may act as chelating agents which prevent precipitation of aluminium at physiological pH values. This hypothesis is used as the basis for an explanation of the different responses shown by various ecological groups of plants to excess aluminium.

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PRE-SOWING GAMMA IRRADIATION OF COTTON SEEDS FROM A POINT SOURCE OF Cs^{137} AND ITS EFFECT ON EMERGENCE, GROWTH AND YIELD

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INTRODUCTION

The biological radiation effects have recently attained increasing interest. The ever growing availability of the various sources of ionizing radiation had led to the growth of research on the effect of radiation on plants. At present there are large potentialities of application of such radiation in agriculture and plant breeding programs. Such potentialities have been evaluated by many authors, *e.g.* Tolbert and Pearson [15], and Singleton [13], It has been already established that under the influence of irradiation, physiological as well as genetical effects could be induced in the treated plants.

Previous work with ionizing radiation has already shown that growth inhibition or stunting is a very common effect. On the other hand, benificial effects of γ-irradiation on plants are still very much disputed (Johnson [10], and Sax [12]).

The work here described was carried out to elucidate the effect of γ-irradiation of cotton seeds from radioactive Cs^{137} source on emergence, growth, flowering, and yield.

MATERIAL AND METHODS

Dry seeds of a pure strain of the Egyptian cotton (*Gossypium barbadense* var. Karnak) were used in this investigation. The radioactive source was supplied by the courtesy of the Egyptian Atomic Energy Commission. It has a strength of 1.22 Curies with a discrete energy of 0.661 Mev and giving a dose rate of 4428.6 r/hour at 1 cm distance. Seeds were placed in circles around the source; the radii of the circles were 2.5, 5, 7, 9.5, and 12 cm. Seeds

were subjected continuously to irradiation for 38 hours. The dose rates and total doses in the different treatments are recorded in Table 1.

TABLE I

Dose rates and total doses of γ-radiation to which the differently treated cotton seeds are subjected during the 38 hours of exposure

Treatment	Distance from source, cm	Dose rate r/h	Total dose r
Control			
Treatment I	12	30.75	1168.6
,, II	9.5	49.06	1864.3
,, III	7	90.36	3433.6
,, IV	5	177.12	6729.9
,, V	2.5	708.48	26922.0

Seeds were sown on the 23rd of March, 1957, in the Botany Department Experimental field. Sowing was made immediately after the exposure period and the seeds were distributed in shallow holes at the rate of 70 seeds per row. Five replicates of each treatment were made. Controls of non-irradiated seeds were kept. Emergence counts were made after 3 weeks from sowing. Growth as indicated by the length of shoot system, fresh and dry weights of the stems, roots, and leaves of the experimental plants was determined after 3, 6 and 11 weeks. Maximum flowering and boll formation was studied after 12 weeks from sowing. The yield was determined on the 20th of September, 1957.

RESULTS

1. *Emergence of seedlings*

Irradiation of dry seeds before sowing appreciably affected their percentage emergence. Increasing the γ-dose caused a considerable reduction in emergence. Thus, only 9 per cent of the seeds, compared to the controls emerged when irradiated with 26.92 kr.

The radiation effect on emergence was retained to the seed crop of the experimental plants. Emergence of cotton seeds of the 1957 crop was tested in the summer of 1958. Seedlings of the latter sowings still showed an increasing tendency for reduced emergence corresponding to the dose given to the initial seeds. Thus, emergence of seedlings from the crops of the 26.92 and 6.73 kr-irradiated seeds amounted to about 25 per cent (23 and 28 per cent respectively) of the controls. The respective values for 3.43 kr, 1.86 kr, and 1.17 kr were 51 per cent, 61 per cent, and 67 per cent of controls.

2. *Growth and development*

Observations on growth of cotton plants were made at, more or less, regular intervals. Data on the height of shoot, number and length of internodes, number of leaves and of lateral branches, number of flower buds, flowers and bolls as well as fresh- and dry-weight determinations were taken.

Fig. 1. Photograph of irradiated seed cotton plants (6 weeks old) showing variation in growth. *From left to right:* Control, 26.92 kr, 6.73 kr, 3.43 kr, 1.86 kr, and 1.17 kr doses)

Figs. 1 and 2 show that high doses of radiation caused marked stunting and inhibition of growth while plants resulting from seeds exposed to low doses were, more or less, similar to the controls. Plants with a 26.92-kr pre-sowing dose were seriously dwarfed and deformed. On examination of the small number of plants surviving

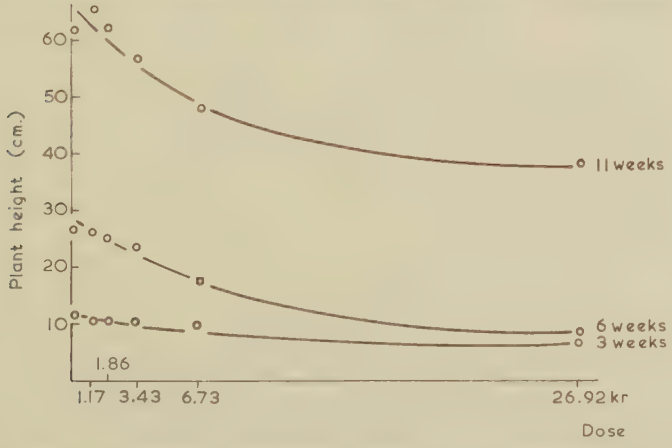

Fig. 2. Effect of different irradiation dosage on the growth of shoot after 3, 6, and 11 weeks. (Expressed as increase in height of shoots in cm).

the 26.92-kr treatments at the end of 11 weeks it was observed that only a few of them reached half the height of the controls, while the others did not show much growth from the 6-weeks stage. The majority of plants, instead of having one main stem, appeared with two axes; this being due to deformation of the growing point and the development of two basal branches from axillary buds.

Leaves of high-dosage irradiated plants were smaller, thicker, wrinkled, and more leathery than those of the controls and some of them showed asymmetrical development of leaf blades.

The number of internodes is significantly reduced in cases of 26.92-kr and 6.73-kr irradiation treatments. The mean number of internodes after 11 weeks was 17 in the control samples, 10 in the 26.92-kr, 11 in the 6.73-kr irradiated samples and around 16 in the other cases.

The number of lateral branches (total and flowering) showed slight variation from the controls in all treatments except 26.92-kr and 6.73-kr irradiations where significant reduction occurred.

Inhibition of growth could also be traced in the fresh and dry weights of shoots and roots. Figs. 3 and 4 show that in all cases except the 26.92-kr irradiation, the increase in dry weight is initially very rapid, but slows down towards maturity. In the case of 26.92-kr irradiation there is an initial increase in the dry weight percentage followed by a gradual decrease. This would indicate that the ex-

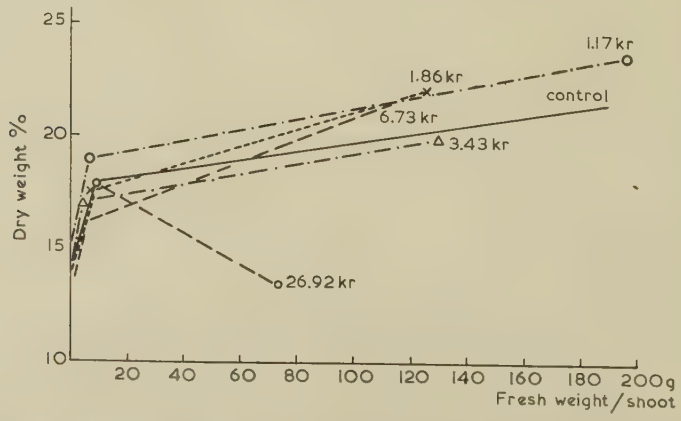

Fig. 3. The variation of the shoot dry weight (percentage of fresh weight) in the different irradiation treatments in relation to the fresh weight per shoot after 3, 6, and 11 weeks from sowing.

perimental plants have a lower rate of gain in weight through processes of assimilation than in other cases.

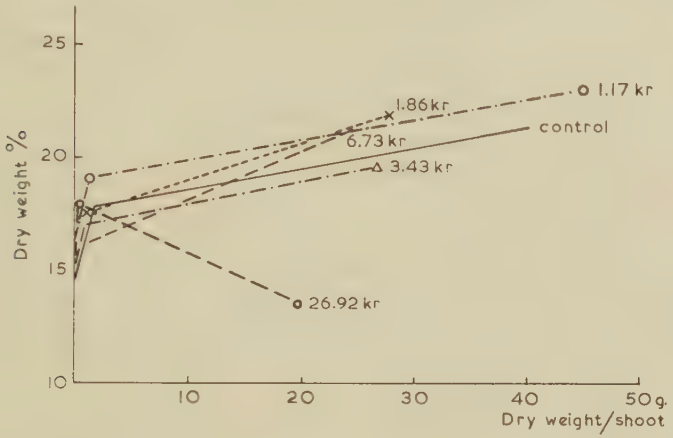

Fig. 4. The variation of the shoot dry weight (percentage of fresh weight) in the different irradiation treatments in relation to the dry weight per shoot after 3, 6, and 11 weeks from sowing.

The root : shoot ratio did not show much variation in the different treatments nor did it show significant variation with age. This means that the inhibiting effect of irradiation is almost the same on shoot as on root.

The dry-weight percentage (of total dry matter) was higher in the leaves and lower in the stems of the 26.92-kr and 6.73-kr treatments than in the controls (Fig. 5).

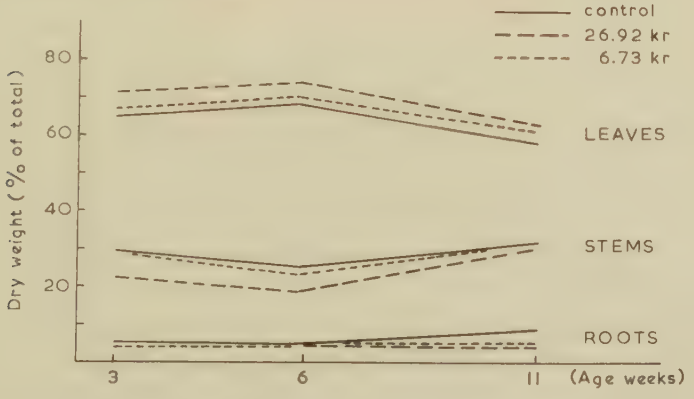

Fig. 5. The distribution of dry matter in stem, leaves, and roots in control, 26.92-kr and 6.73-kr irradiation treatments.

3. *Anatomy*

An observation of interest was that the leaves of plants with high dosages were thicker than those of the controls. The thickness of the leaf in the case of 26.92 kr amounted to 1.21 mm as compared to 0,75 mm in the case of 6.73 kr, and 0.33 mm in case of control. Examination of sections in leaves have shown that there is multiplication of the palisade layer and enlargement of spongy cells with an increased dosage of irradiation.

This observation is in agreement with that reported by Sparrow and Gunckel [14] in *Antirrhinum majus* under chronic γ-irradiation, that with increasing dosage of γ-rays to 600 r per day there was progressive thickness of the leaf up to at least three times that of control. The major part of increase in thickness was due to repeated cell division in the palisade layer.

4. *Cytology*

Cytological preparations of root tips did not reveal significant variations in the different treatments as compared with the controls *. No chromosome doubling or breakage was observed.

5. *Flowering and boll formation*

Flowering was retarded by high dosage whereas low dosage induced no wide difference from controls. Fig. 6 shows that the

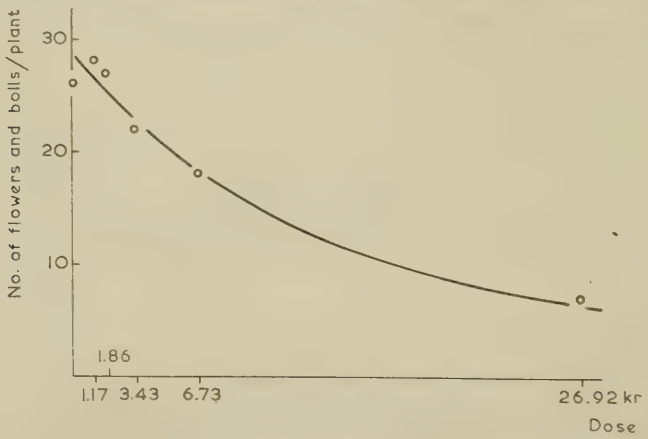

Fig. 6. The mean number of flowers and bolls on cotton plant (12 weeks old) in relation to irradiation dosage.

* The authors are indebted to Dr. H. Hakim for her help in investigating and commenting upon the cytological preparations referred to herein.

number of flowers and bolls is diminished with the increase in dosage. It is to be noticed that the curve (Fig. 6) shows similar trend to the curve representing the relation between dosage and growth (Fig. 2). In this connection it may be mentioned that in 1919 Balls [1] (as quoted by Brown [2]) realized that not only flowering follows the growth, but that if both are plotted the two curves coincide closely.

No appreciable effect of irradiation on boll shedding was observed.

6. *Fertility*

Fertility tests were carried out by examining lactophenol blue-stained preparations of pollen grains of the experimental plants. These tests showed that there existed a percentage of reduced size grains which are morphologically abnormal. This amounted to 17 per cent in the case of 26.92-kr and 14 per cent in case of 6.73-kr irradiation. In other cases values less than 8 per cent were found. Morphologically abnormal grains were taken by many authors (*e.g.* Nybom [11]), as indicating reduction in fertility.

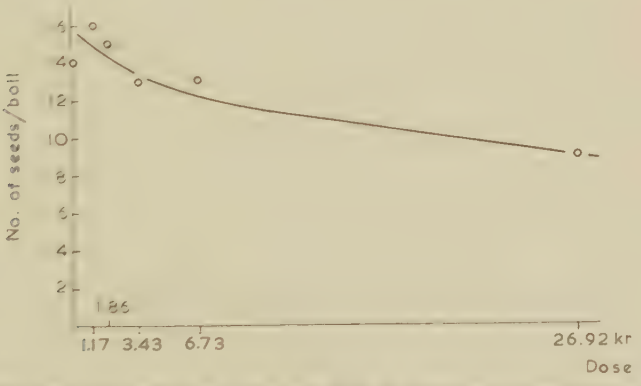

Fig. 7. The mean number of seeds per boll in relation to irradiation dosage.

The degree of seed setting may be taken as an indication of fertility. The mean number of seeds per boll was determined for the plants which had received the different irradiation doses. The results are shown in Fig. 7. Differences between controls and plants with radiation doses lower than 26.92 kr were rather slight but in the latter case fertility was significantly reduced (9 seeds per boll with treated plants as compared to 14 seeds in case of controls).

7. *Yield*

The effect of irradiation on the yield of seed cotton, lint and fuzzy seed is quite clear (Fig. 8). The yield has been reduced considerably by the high irradiation dosage (only 33 per cent of control in case of 26.92-kr dosage). Suppression of seed cotton, seeds, and lint followed almost the same trend. No change in the length, strength, colour, and luster of tho cotton fibres was detected under the various experimental conditions except in the case of 26.92-kr irradiation where relatively bad quality of lint was produced.

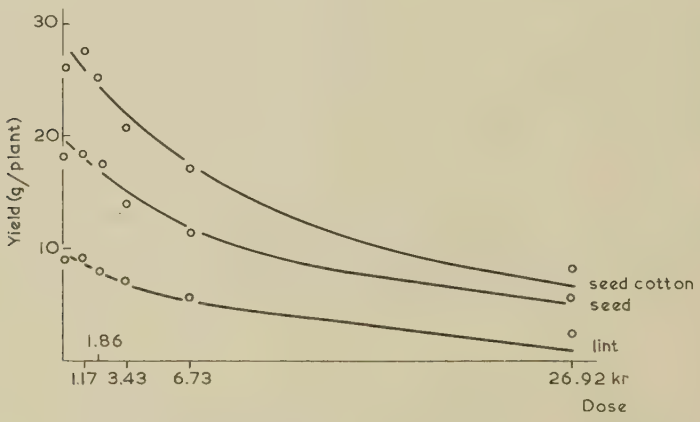

Fig. 8. Yield of seed cotton, seed, and lint per plant in relation to irradiation dosage.

DISCUSSION

The results of this investigation clearly show that irradiation of cotton seeds with γ-rays (doses of 1.17 to 26.92 kr) has a profound influence on emergence, growth, flowering, and yield. With increasing the radiation dose, emergence was suppressed and growth inhibited. Flowering was retarded and the number of flowers and fruits was reduced. Serious reduction in the yield occurred in case of high dosage. Similar inhibitory effects of radiation on growth, mottling of leaves, and many different types of leaf deformations under the influence of ionizing radiations were obtained by many authors (*e.g.*, Granhall *et al.*[8]; Ehrenberg and Granhall [4], Granhall *et al.*[7], and Nybom [11]).

On the other hand, there are in the literature recorded cases of stimulating effects of radioactivity on growth and yield. Thus

Johnson [10] recorded a stimulating effect of radiation on flowering and ripening of Kalanchoe plants. Sparrow and Gunckel [14] have shown that some plants of *Antirrhinum majus* and Nicotiana grown in γ-field grew, at certain dose rates, much taller than normally. More commonly, the growth of plants exposed to chronic γ-irradiation is inversely proportional to the dose rate. Carter [3] has reported that ionizing radiations speed up the supply of new mutations and though most of these are deleterious (from the viewpoint of the breeder as well as of the plant), a few may give agriculturally desirable characteristics. In our experiments no stimulating effect of the radioactivity has been observed.

The effect of radiation on living plants is very much disputed in the literature. However, evidence is more in favour of the fact that the effect of densely ionizing radiation, *e.g.* neutrons, on plants is chiefly localized in the cell nuclei, whereas sparsely ionizing radiation such as γ- and X-rays produce their effects through the action on cytoplasm (Ehrenberg and Nybom [5], and Ehrenberg and Sealand [6]). Neutrons cause chromosome injury manifested as rearrangements, sterility, or mutations. The results of the present investigation revealed no changes in the chromosome structures of the irradiated cotton plants when compared with the control ones.

Nybom [11] believes that the size of chromosomes plays an important role in the specific sensitivity towards irradiation and that the effects of the radiation on the chromosomes in perhaps decisive for the damage leading to induced growth inhibition, abnormal development, and death. Sparrow and Gunckel [14] stated that plants with smaller chromosomes tend to be less sensitive than those with large chromosomes. In this connection it may be noted that cotton is characterized by small-sized chromosomes and thus according to Nybom's and Sparrow and Gunckel's views, cotton is of relatively low radiosensitivity. The effects observed on the experimental plants would, most probably, be due to physiological disturbances or injuries.

The strong sterilizing effect of γ-rays on cotton is quite evident. In case of 26.92-kr irradiated seeds, emergence was reduced to 9 per cent of the controls and each of the surviving plants gave 8 bolls with 9 seeds each as compared with 26 bolls with 14 seeds each in the non-irradiated plants. Thus seed setting per plant is diminished to 19.7 per cent of that of the control.

Sterility may be induced through sterile pollen or aborting ovules. The low percentage of morphologically abnormal pollen grains could not be responsible for the observed reduced degree of fertility in case of 26.92 kr irradiated plants. Such reduction in fertility may be explicable on the basis of the fact that the normal appearance of the pollen grain may not be taken as an indication of its fertility. Alternatively, there may be ovule abortion in effect of irradiation damage. Similar proposals were suggested earlier by Nybom [4] who reported that flax, barley, and beans showed increased sterility with increasing dose of x-irradiation. Flax was the most resistent and beans the least.

SUMMARY

The effect of pre-sowing irradiation of cotton seeds from a point source of Cs^{137} on emergence, growth, flowering, and yield was studied. High radiation doses caused reduction of emergence, inhibition of growth, cessation of flowering, and reduction in number of flowers and bolls produced by the plant. The yield was greatly reduced under high dosages and all irradiated lots of the control plants under low dosages. Pollen caused a different degree of sterility.

No significant stimulating effect on growth and development was detected when the dry cotton seeds were subjected to irradiation doses of 1.35 to 26.92 kr.

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SOME PHYSICAL PROPERTIES OF POT-PLANT COMPOSTS AND THEIR EFFECT ON PLANT GROWTH

I. BULKY PHYSICAL CONDITIONERS

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INTRODUCTION

The effect of various physical properties of soil on the growth of plants in the field has received wide consideration. There is, however, very little information regarding the desired physical properties of pot-plant composts.

The use of pots or similar containers changes the normal physical relationship between root and substrate in the following principal ways.

1. The small volume of the pot leads to a high root concentration with consequent demands for a high rate of oxygen supply and carbon dioxide removal.

2. The large quantity of water required to sustain the high growth rates normally encountered under glass must be available from within the very restricted volume of soil.

3. The shallow depth of the container with its low tension head causes impeded drainage with risk of water-logging, especially under winter conditions.

4. The high frequency of watering renders the substrate liable to leaching, especially if it is open structured.

For these reasons it is generally accepted that 'conditioners' must be used to improve the physical properties of soils before they are used for pot-plant cultivation. The conditioner should be a standard-

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ized material, readily available, free from pathogens, and without phytotoxicity if steam sterilized. Both peat and grit meet these requirements and have been extensively used, (Longley [8], Ray and Shanks [10], Brase [3], McCool [9], Lawrence and Newell [7], Fruhstorfer [6], Baker [1], Wild [11], and Baver [2].) Because of the paucity of physical data on pot-plant composts an experiment was designed to determine, in terms of both the physical effect and the response of the plant, the extent to which loams having different structural properties need conditioning and the effect of peat and grit when added to the soils in different amounts (mixtures).

MATERIALS AND METHODS

Four soils were selected which, from a visual assessment, exhibited a range of structural properties, *viz* 1) chalky boulder clay; 2) brick earth; 3) sandy soil; 4) fine sand. The first three are Bayfordbury soils and the fourth came from Spalding in Lincolnshire. The mechanical analyses (per cent) of these soils based on the pipette method are presented below.

Analysis	Soil			
	Chalky boulder clay	Brick-earth	Sandy soil	Fine sand
Clay	32.7	21.2	12.2	12.0
Silt	16.6	13.9	13.0	6.5
Fine sand	28.9	49.5	41.3	73.0
Coarse sand	9.7	7.7	26.8	0.6
pH	7.2	5.9	5.7	6.3
Total nitrogen %	0.236	0.223	0.189	0.172

To determine desorption (pF) curves, undisturbed cores were used, 2 cm deep and 5.1 cm diameter taken from a sample of each mixture which had been loosely placed in a box on the glasshouse bench and watered daily for six weeks from a watering can fitted with a 'rose'. This allowed each mixture to stabilize at its natural compression. The cores were placed on plates of sintered glass (porosity No. 5) similar to that described by Croney *et al* [5]. Each curve represents a mean value of four determinations.

Similar cores were used for permeability determinations made with a constant head of 2 cm of water. The 2 cm deep cores were placed on a perforated zinc sheet within a glass funnel, slowly flooded, and then kept covered with water until the permeability rate was determined. The permeability figure for each mixture in Table 4 is the mean of six determinations, each determination consisting of ten observations of the flow rate at two-minute intervals.

Wilting-point determinations were made using standard techniques with

sunflowers, a correction being applied for the estimated amount of water in the roots. Macropore content was taken as being the volume of the sample drained at 40 cm water tension (pF 1.6); and available micro-pore content as the volume between pF 1.6 and 4.2. Air capacity and water retention were determined in cans 10 cm deep, having a perforated zinc base covered with a disc of filter paper (Whatman No. 4). The mixture was loosely poured into the cans and after daily watering for six weeks from a watering can fitted with a 'rose', the cans were slowly flooded and allowed to stand overnight. They were then fitted with lids to prevent evaporation and allowed to drain for 48 hours. The specific gravity of each mixture was determined, in triplicate, by the British Standards Institution [4] procedure.

Two bulky conditioners were used, *viz*, a granite grit and a sphagnum-moss peat of pH 3.8, each having the following sieve-size analysis (per cent by weight).

Sieve size	Peat	Grit
> 5.0 mm	20.8	0.6
5–3 mm	23.6	4.8
3–2 mm	13.8	21.4
2–1 mm	17.4	44.7
1–0.5 mm	14.4	23.8
< 0.5 mm	10.2	5.3

These materials were used to make the following mixtures (volume basis).

Mixture	Soil	Peat	Grit
A	100	—	—
B	60	40	—
C	60		40
D	60	25	15

The four mixtures, used with the four soils, gave sixteen different treatments which will be denoted by the numerals 1 to 4 for the soils and the letters A to D for the mixtures. The pH of soils 2 and 3 was adjusted to 6.3 before starting the experiment. Because of the large number and size of the tomato plants grown, dry-matter determinations were restricted to a random sample of each treatment.

EXPERIMENTAL

Two plants having different growth rates were chosen, namely, *Lycopersicon esculentum* 'Potentate' and *Antirrhinum orontium*, both being inbreeders and relatively uniform in growth.

Tomato

(i) Growth. The tomatoes were pricked out on February 6th and grown in 5-inch pots in randomised-block design with 18 plants per

treatment. These were scored for fresh weight and also for the length of the period between pricking out and the opening of the first flower (days to anthesis). In addition, 6 plants of each treatment were grown on in 10-inch pots and the yield of fruit for the first four inflorescences recorded. The large basic differences in chemical fertility created by the conditioners could be expected materially to influence the results and to minimise this, a nutrient solution having NO_3 – 413, NH_4 – 27, P – 31, K – 117, and Mg – 24 ppm was applied at each irrigation. All treatments had superphosphate added at the rate of 1 g per litre (or JIP1 * strength) of mixture before potting up.

The steam sterilisation of the chalky boulder-clay soil caused a pronounced check to seedling tomatoes and the death of seedling antirrhinums. This sterilising toxicity was confined to treatments in which no peat was used, *i.e.* 1A and 1C. Treatments having peat included (*i.e.* 1B and 1D) showed no signs of the sterilising toxicity and growth was apparently normal. Because of this sterilising toxicity the results for the boulder-clay series of mixtures are not included in this paper.

The fresh weight and per cent dry matter of the tomato plants in the different treatments are given in Table 1. The admixture of

TABLE 1

Fresh weight and % dry matter of tomato
Treatment means
Dry-matter per cent in parentheses.

Soil	Treatment			
	A	B	C	D
[illegible]	[illegible]	[illegible]	52.4	59.4
	[illegible]	[illegible]	(12.88)	(12.19)
[illegible]	[illegible]	[illegible]	58.0	77.4
	[illegible]	[illegible]	(11.53)	[illegible]
[illegible]	[illegible]	73.6	31.4	[illegible]
	[illegible]	(12.41)	(12.41)	[illegible]

L.S.D. at 5 per cent = 5.5 215 d.f.

40 per cent peat gave an increase in weight of approximately 10 g over the control, 25 per cent peat increased the weight by approximately 6 g, whilst 40 per cent grit decreased the weight by approximately 10 g.

* JIP1 = the John Innes Potting Compost with one dose of base fertilizer.

(ii) Anthesis. Tomatoes in substrates which had peat included flowered 2.0 days earlier than the controls at the first inflorescence (L.S.D. at P 5% = 0.5 day). For the first four inflorescences those treatments having peat included flowered 2.6 days earlier than the controls and the treatments having grit included flowered 1.7 days later than the controls (L.S.D. at P 5% = 0.9 days).

(iii) Fruiting. The effect of the different conditioners on fruit yield is presented in Table 2. Early yield is defined as the cumulative

TABLE 2

Cumulative tomato yield for early and total crop (mean values, g per plant)

Soil	Treatment			
	A	B	C	D
Early crop				
Brick earth	85.90	87.28	84.00	97.28
Sandy soil	95.53	95.93	77.93	98.42
Fine sand	80.48	90.03	71.77	82.92
L.S.D. at 5 per cent = 10.4				
Total crop				
Brick earth	131.0	123.7	124.6	130.6
Sandy soil	134.0	132.9	126.9	127.8
Fine sand	111.9	113.8	104.7	113.7
L.S.D. at 5 per cent = 14.95				

yield for the first four weeks of picking, which commenced on June 3rd. The beneficial effects of the peat and the delaying or inhibiting effect of the grit in the mixtures are not as consistently shown in the early yield as they were in either the vegetative growth or days to anthesis. There are no significant differences in total yield due to the conditioners. The weights of total fruit in all treatments in the fine sand series (soil No. 4) are significantly lower than either of the other two soil series.

Antirrhinum orontium

This species was chosen to enable the effects of the various conditioners to be studied during the winter months when growth rates are low and a change in optimal physical properties, *e.g.* better drainage, might be required. Seedlings were pricked-out on October 1st., 24 per treatment. The nutrient-solution strength was reduced to NO_3 – 248, P – 31, K – 78, Mg – 24 ppm. The results of this

experiment are presented in Table 3. Peat gave a significant increase in growth with all soils and grit a significant increase with the sandy soil. The fine-sand soil was the most responsive one to the addition of peat, but unlike the other two soils, gave a significant reduction in growth with 40 per cent grit (treatment No. 4C).

TABLE 3

Soil	Dry weight (g) of Antirrhinum			
	Treatment			
	A	B	C	D
Brick earth	0.778	0.907	0.872	1.099
Sandy soil	0.911	1.145	1.113	1.212
Fine sand	0.404	0.967	0.282	0.874
L.S.D. at 5 per cent = 0.12 g				

The fresh weights of tomato and Antirrhinum grown in the different treatments were examined to determine the extent to which similar growth responses, if any, were obtained with these two species which had markedly different growth rates. It was found that comparable treatment fresh weights of tomato and Antirrhinum were positively correlated ($r = +0.78$, $P = 0.01$). The growth rate * for tomato was 0.074 and for the Antirrhinum 0.035.

Physical properties of mixtures

The physical properties of each treatment are presented in summarised form in Table 4. The wide fluctuations in volume weights of the mixtures invalidates any comparison of water retention *etc.*, on a weight basis and for this reason all physical data are presented on a volume basis. In all cases the effect of 40 per cent of peat was to increase the total pore space, the macro-pore content, the air capacity, the total available pores and available water. Peat increased the permeability most in the fine sand and least in the brick earth.

The addition of 40 per cent of grit increased the macro-pore content and permeability rate but decreased the total porosity, the

* The growth rates were calculated from:

$$\frac{e^{\log \text{ mean fresh weight for all treatments}}}{\text{number of days from pricking-out to cutting}}$$

TABLE 4

Some physical properties of the soils and mixtures

	Treatment												L.S.D.
	2A	2B	2C	2D	3A	3B	3C	3D	4A	4B	4C	4D	$P=0.05$
Total porosity % .	64.8	73.9	56.5	67.7	62.8	75.2	58.9	69.2	51.1	68.9	45.9	59.0	1.79
Macro pores (pF 0–1.6)	31.5	37.4	34.1	38.7	30.2	41.7	34.9	40.5	8.0	24.4	14.7	20.9	—
Air capacity . . .	13.3	15.0	12.8	11.6	8.3	13.1	8.8	12.3	7.5	12.9	8.2	9.8	2.95
Wilting point (% volume basis) . . .	14.2	10.6	7.2	9.5	9.4	9.0	6.8	8.3	9.5	9.5	6.4	8.9	1.02
Available micro pores (pF 1.6–4.2) .	19.1	25.9	15.2	20.0	23.2	24.5	17.2	20.4	33.6	35.0	24.8	29.2	—
*Available water capacity	37.3	48.3	36.5	47.1	45.1	53.1	43.3	48.6	34.1	46.5	31.3	40.3	—
Permeability (ml/min)	212	241	239	262	78	139	185	177	10	117	51	94	46.3
Volume weight . .	0.88	0.62	1.15	0.83	0.89	0.58	1.06	0.77	1.25	0.76	1.41	1.04	—
Specific gravity . .	2.52	2.40	2.64	2.58	2.49	2.40	2.63	2.54	2.55	2.44	2.63	2.55	—

* Available water capacity = the difference in volume of water at air capacity and the volume at wilting point.

available micro pores and available water capacity. There was no significant effect on the air capacity. The effect of the addition of 25 per cent peat and 15 per cent grit was to give approximately intermediate values to either of the conditioners when used separately.

The pF or desorption curves of the three soil series are shown in Figs. 1, 2 and 3. Whilst the reduction in total pore content resulting from the addition of grit and the increase in total pore content resulting from the addition of peat is clearly shown for all soils, the change in total and macro-pore content of the fine sand resulting from the addition of peat is most apparent. Correlation of the various physical values with those for plant growth and development are presented in Table 5.

The effect of the grit particle size on some physical properties of the composts was examined by using two sizes of separates, 0.5–1.0 mm and 3.0–5.0 mm. These would approximate to fine and coarse grits as used in commercial practice. Desorption curves, with four replicates per treatment, showed that the particle size of the grit had no practical effect when used in mixtures having 60 per cent soil, 25 per cent peat and 15 per cent grit. The differences in total and macro porosity due to the size of the grit were less than 3 per cent with all soils.

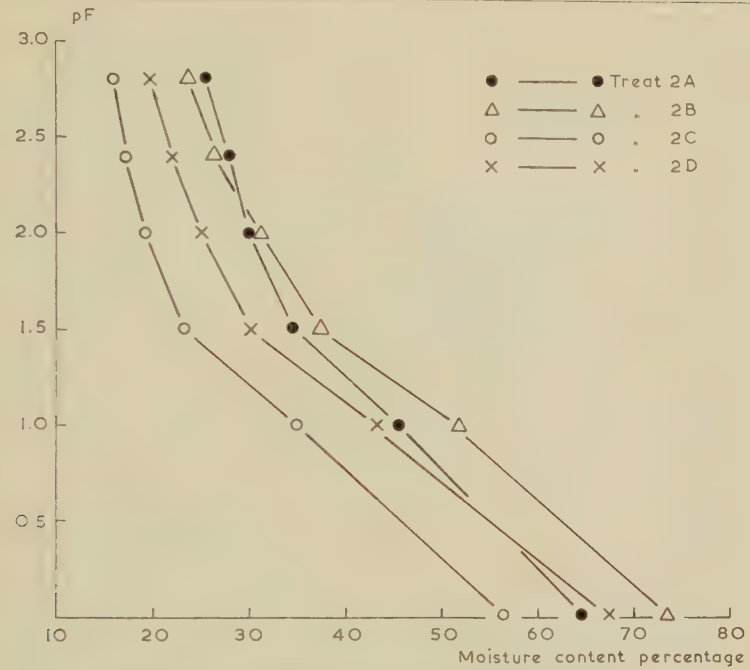

Fig. 1. Desorption curves. Brick-earth series.

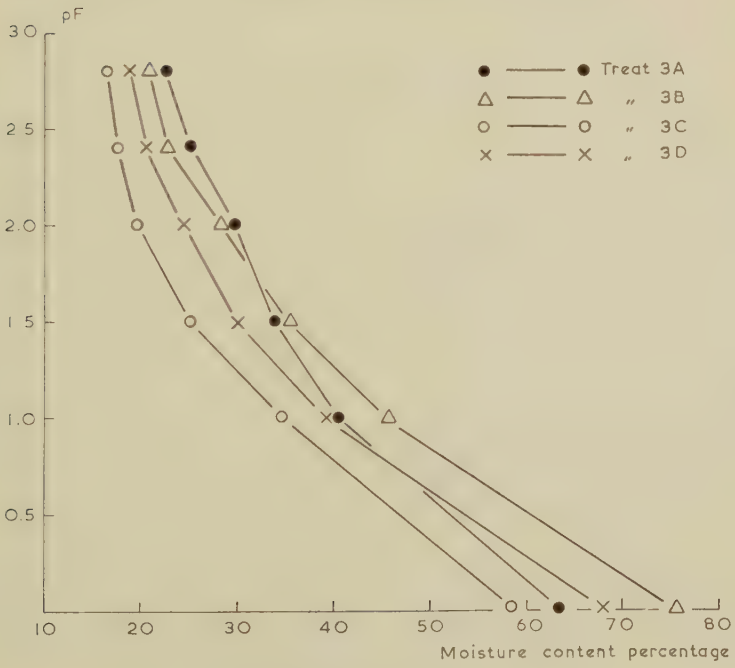

Fig. 2. Desorption curves. Sandy-soil series.

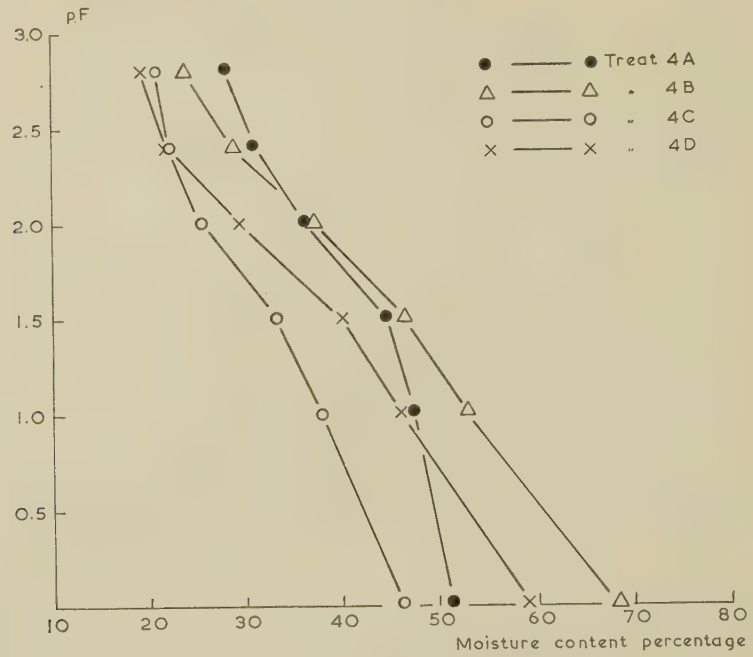

Fig. 3. Desorption curves. Fine-sand series.

TABLE 5

Correlation coefficients		
Correlation	r value	P value
Tomato		
Fresh weight and air capacity	+ 0.51	N.S.
Fresh weight and macro pores	+ 0.79	0.01
Fresh weight and permeability log 10	+ 0.51	N.S.
Anthesis 1st inflorescence and air capacity	− 0.71	0.01
Anthesis 1st four inflorescences and air capacity	− 0.61	0.05
Anthesis 1st inflorescence and macro pores	− 0.30	N.S.
Anthesis 1st inflorescence and permeability log 10	− 0.25	N.S.
Antirrhinum		
Fresh weight and air capacity	+ 0.40	N.S.
Fresh weight and macro pores	+ 0.85	0.001
Fresh weight and permeability log 10	+ 0.71	0.001

DISCUSSION

It is evident from the data presented that certain of the physical properties of the soils, principally volume-weight, total pore space, macro-pores and permeability, have a much higher value than

those obtained from soils in the field. This difference is due to the preparatory treatment of the soils prior to sterilization, *i.e.* mechanical shredding, and the subsequent absence of compression other than that caused by watering.

Peat was more effective than grit in enhancing these properties. Thus the fine sand which, from a visual assessment and the pF curve, ha dthe poorest structure, gave the largest response to peat, and the brick earth, which had the best structure, gave the smallest response.

Grit increased the macro-pores and the permeability, but decreased the total-pore and available-water content and gave no significant change in the air capacity. Thus from general physical considerations grit would appear inferior to peat as a conditioner for pot-plant mixtures. Also the particle size of the grit over the range 0.5–1.0 to 3.0 5.0 mm appears to have no significant effect on total or macro-pore content.

Considering the effect of the conditioners in terms of plant growth, peat gave improved growth (weight) and earlier flowering with tomato and improved growth with Antirrhinum. Also it eliminated toxicity in the boulder-clay soil caused by steam sterilization.

Whilst the macro-pore content of the various treatments gave the best physical assessment of ultimate plant growth it should be noted that in six treatments the increase in macro-pore content was achieved by the use of peat and in three treatments a small increase in macro-pore content was achieved by the use of grit. It cannot therefore be assumed that the improvement in plant growth was due to the increased macro-pore content rather than to some inherent property of the peat. Peat, unlike grit, is not an inert conditioner. In addition to the physical changes it induces peat also influences the chemical and biological properties of the mixture. Further, various growth substances have been identified in peat by several workers and these may play a part. The extent to which these various factors have influenced plant growth cannot be assessed from the data presented. The physical changes induced by the addition of peat would not appear sufficient to explain the elimination of the toxicity in the boulder-clay soil due to steam sterilizing.

From a practical point of view it will be seen that the effect of the peat in the mixtures was greater than that of the grit. Also the grading of the grit over the range used appeared to be unimportant.

In this study, normal densities for all treatments have been used.

The effect of compaction, such as occurs in the 'potting-on' of plants, will be presented in a subsequent paper.

SUMMARY

The effect of peat and grit on the physical properties of composts made from soils of different structural properties has been examined. Peat was beneficial in giving improved physical properties to the composts, better vegetative growth of tomato and Antirrhinum and also earlier flowering with tomato. It had, however, no significant effect on the total yield of tomato fruit. Peat also eliminated a sterilizing toxicity in a chalky boulder clay.

Measurement of macro-pore volume gave the best physical assessment of the mixtures with respect to vegetative growth, but the extent to which the improved growth was dependent on the physical property or to some inherent property of the peat has not been determined.

Grit was not such a good physical conditioner from either a plant growth or physical point of view as peat. The size of the grit over the range 0.5–1.0 to 3.0–5.0 mm did not cause any significant change in the physical properties of mixtures when used in the proportions 60 soil, 25 well-granulated peat, 15 grit.

ACKNOWLEDGMENTS

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ORGANIC MATTER AND NUTRIENT CYCLES UNDER MOIST TROPICAL FOREST

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INTRODUCTION

In a recent paper in this journal, Greenland and Kowal [3] described the measurement of the total dry matter and the nutrient capital accumulated in the aerial parts and roots of a mature secondary forest, about 40 years old, at Kade, Ghana. The present paper describes work designed primarily to estimate the rate of turn-over of organic matter and nutrients in the same forest.

The return of organic matter and nutrients from the vegetation to the soil occurs in the following forms:

I. litter fall
II. timber fall
III. root decomposition and nutrient excretion from roots, and, for the nutrients only,
IV. rain wash from the standing vegetation.

In investigations of the nutrient cycle under forest and woodland it has often been assumed that the nutrients in the litter fall represented all or nearly all the loss. The amount and composition of litter fall have been measured in a number of temperate regions, and the results have been summarized by Lutz and Chandler [10]. In the tropics the only data giving both amount and composition are provided by the measurements of Laudelout and Meyer [9] in the Belgian Congo forest.

In addition to the litter fall, in the present work some allowance is made for the timber fall; that is, the dead branches and stems that are not collected in the litter trays. An allowance is also made for addition of organic matter by roots.

The importance of leaching of nutrients from the forest canopy by rain has only recently been recognized. It has for long been known that salts could be washed out of crop plants by rain, but most of the quantitative data on such leaching has been obtained under artificial conditions with detached leaves, and not on intact plants. Stenlid [14] has recently reviewed the literature on this subject. Data for trees are particularly scarce. Tamm [15], in a study of leaching from six deciduous species and two conifers in Sweden, found considerable amounts of K were leached, but there was little loss of Na or Ca. Will [17] has recently reported the results of a comprehensive study of litter fall and rain leaching from exotic conifers in New Zealand. At least two thirds of the K reaching the soil under radiata pine and Douglas fir, and half the P under Douglas fir do so in the rain water. Leaching of Ca and Na from the needles was slight, except for Na on one site high in exchangeable Na.

Though the forest at Kade is certainly not primary (such examples are rare and inaccessible these days) there is good evidence that little or no further accumulation of organic matter or nutrients is taking place in it. As Greenland and Kowal have indicated, the basal area at breast height of the trees in the example studied is 145 sq. ft. per acre. According to Dawkins [1] this is the value attained by a primary tropical high forest under favourable conditions. Thus if there is no further net growth or accumulation of nutrients in this forest the rate of litter and timber fall represents the total production of organic matter. The total rate of loss of nutrients from the vegetation will also be a measure of their rate of uptake from the soil.

THE SITE

A description of the forest and soil, and some details of location and climate have already been given by Greenland and Kowal [3]. It is sufficient to note here that the forest is in the ecotone between the moist semi-deciduous and the moist evergreen forest. It contains a wide range of species assorted among a fairly open shrub layer, a dense lower storey extending from some 15 to 50 ft., and a more scattered upper storey extending to 130 ft. with occasional massive-crowned emergents rising through it. The present work was carried out in the undisturbed forest on either side of the $1\frac{1}{2}$ acre block that was felled for estimating the nutrients stored, and was to all appearances identical.

The rainfall, which averages 65 inches p.a., is concentrated in a major rainy season from March to July and a minor season from September to December. During the main dry season centred on January there is often a lot of dust in the air carried by the 'Harmattan' wind from the desert regions far to the North, and this is likely to affect the mineral content of the rain-water. During the rest of the year the prevailing wind blows off the sea, which lies 60 miles to the South.

EXPERIMENTAL METHODS

Litter was collected in six nylon mesh trays measuring 3 ft. × 6 ft., which were raised one foot from the ground. The litter falling in these trays was collected every two weeks over a period of a year. After each collection the tray was moved to a fresh site, in order, over the year, to increase the effective sampling of litter from the extremely heterogeneous vegetation. The litter was air dried, weighed, and bulked. At the end of the year it was thoroughly mixed, sampled in triplicate, and the samples ground for analysis. Litter was collected from May 1958 to April 1959.

Rainfall and leaf drips were collected in 8-in. diameter copper rain gauges fitted with a nylon mesh screen and a plug of cotton wool at the base of the funnel. There were six gauges under the forest and three in the open clearing. The gauges were emptied every week and the bulked samples from each gauge separately analysed every month. During the first six months of the experiment the nylon screens in the gauges were cleared of any fallen litter every week. In the second six months the number of gauges under forest was increased to eight. Four of them were cleared every day, and the other four every two weeks — the same interval as the litter trays were cleared. Over this six month period the difference in the composition of each set of four gauges was insignificant. This shows that the substantial amounts of nutrients collected in the gauges under the forest were little affected by leachings from the small amounts of litter held up by the nylon screens of the gauges. It also indicates that the litter lost only small amounts of nutrients while on the trays.

ANALYTICAL METHODS

N, P, and K in the litter were analysed by the method of Hutton and Nye [6], which uses a sulphuric acid and selenium digestion. Ca and Mg were determined following a digestion with perchloric and nitric acids, since the highly siliceous residue was found to retain small amounts of these elements in a sulphuric acid digest. Ca in the digest solution was determined by titration with versene using Patton and Reader's indicator. Ca and Mg together were also determined by titration with versene using Eriochrome Black T as indicator. The Mg value, determined by difference, was checked by a Titan Yellow method.

Rain-water samples were analysed as follows: NH_4-N by micro-Kjeldahl distillation and titration with 0.001 *N* HCl; NO_3-N by the phenol disulphonic acid method; P by the ammonium molybdate — stannous chloride method;

K and Na by EEL flame photometer; Ca and Mg as described for the litter samples; HCO_3 by titration with 0.001 *N* HCl using bromo-phenol blue as indicator; Cl by titration with silver nitrate using potassium chromate as indicator; SO_4 by the turbidimetric method.

EXPERIMENTAL RESULTS

1. *The rate of production and decomposition of litter*

Annual production. The total amount of litter falling during the 12 month period was 9,400 lb. per acre (oven dry). Leaves formed two thirds of the total, the remainder being mainly twigs and small-wood. This result agrees with the amount of 11,000 lb. per acre (dry matter) recorded by Laudelout and Meyer [9] under mixed forest at Yangambi in the Belgian Congo, and the amounts of 7,000 and 9,100 lb. per acre (oven dry) recorded by Jenny *et al.* [7] under rain forest in Columbia.

Seasonal distribution. The cumulative total of the bi-weekly collections of litter through the year is shown in Fig. 1. It bears out the description of this forest as an integrade between the moist evergreen and the moist semi-deciduous forest. Though the fall of

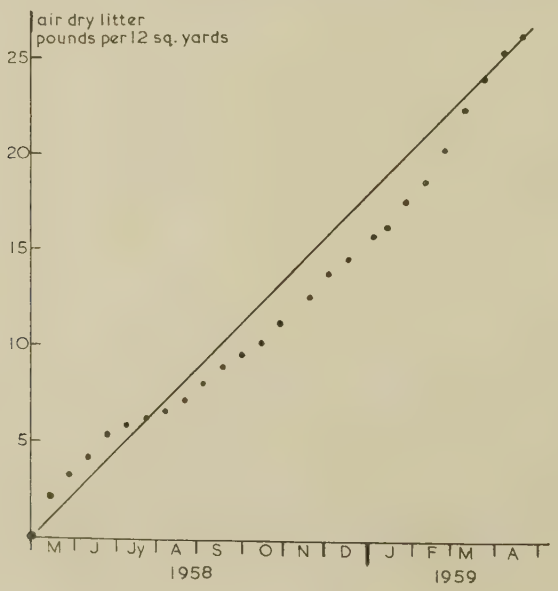

Fig. 1. Litter fall. Cumulative total of 2-weekly collections.

litter is continuous, the influence of the short dry season in January and February may be observed in the relatively high rate of accumulation during February and March. There is a relatively low rate of accumulation from mid-June to mid-August.

The rate of decomposition. Reference is often made to the high rate of decomposition of organic matter in the humid tropics. In the present instance the rate of disappearance of litter from the forest floor can be deduced if the litter fall and the amount of litter on the ground are known. When the litter has built up to an equilibrium value the rate of addition in a small time element dT, will equal the rate of loss over the same period. Hence

$$A \times dT = kL \times dT \qquad (1)$$

where A is the litter fall per annum
L is the amount of litter on the floor
k is the annual decomposition constant.

Greenland and Kowal [3] found the amount of litter on the floor of this forest to be 2,020 lb. per acre (oven dry), a result based on 12 sample plots of 20 sq. yd. each, sampled in late January. The rate of decomposition is therefore $\frac{9{,}400 \times 100}{2{,}020} = 465$ per cent per annum, or 1.3 per cent per day — a very high figure.

Jenny *et al.* [7], who first developed this approach, calculated that the annual decomposition constant of litter in moist evergreen forest in Columbia was 40 to 60 per cent and Laudelout and Meyer [9] obtained a value of 76 per cent for the moist evergreen forest at Yangambi. Both workers used the equation

$$A = k(L + A) \qquad (2)$$

to calculate the decomposition constant. This equation was derived by Jenny for conditions with a marked seasonal litter fall. Its use when A is large compared with L, and when litter fall is continuous, leads to a large error. For instance, using Equation (1), the annual decomposition constant for the Belgian Congo is raised from 76 to 316 per cent, a value in fairly good agreement with that obtained for the forest at Kade.

Jenny *et al.* [7] calculate that the annual decomposition constant for litter under a Californian oak forest is 6 to 12 per cent per

annum. The vastly accelerated rate of decomposition of litter in the tropical forest is most striking. At Kade, the difference is probably due in large measure to the intense activity of myriads of termites and ants, which rapidly comminute the litter so that it is readily attacked by smaller organisms.

Though the rate of destruction of litter under tropical forest is so much greater than under temperate forest, it is worth emphasizing that the humus in this tropical forest has been estimated to decompose at a rate of only about 3 per cent per annum (Greenland and Nye [4]).

Nutrient composition of the litter. The nutrient composition of the whole litter fall, and of the leaf fraction only, is shown

TABLE 1

Nutrient composition of litter (%)							
Place	Forest	Condition	N	P	K	Ca	Mg
Fresh litter							
Kade, Ghana	Moist tropical mixed spp.	Oven dry	1.90	0.069	0.65	1.99	0.43
Yangambi, Belgian Congo	Moist tropical mixed spp.	Dry matter	1.8	0.06	0.39	0.85	0.43
Fresh litter-leaf fraction only							
Kade, Ghana	Average value for for 14 broad-leaved forest spp.	Oven dry	2.10	0.087	1.00	2.02	0.54
Eastern United States		Dry weight	0.68	0.14	0.65	2.07	0.35
Litter on the forest floor							
Kade, Ghana		Oven dry	1.54	0.057	0.45	1.98	0.24
Dead wood							
Kade, Ghana		Oven dry	0.32	0.026	0.05	0.73	0.07

in Table 1. It will be seen that the freshly fallen litter is somewhat richer in N, P, K, and Mg than, and similar in Ca to, the litter of mixed age collected from the forest floor by Greenland and Kowal. The litter collected by Laudelout and Meyer [9] from the moist evergreen forest at Yangambi, Belgian Congo, where the soils are very acid, is considerably poorer in Ca and K.

The leaf fraction of the litter, which forms two thirds of the total, is richer than the remainder in all nutrients except Ca. Its compo-

sition may be compared with that of the leaf litter from temperate broad-leaved forests. For this purpose the mean of average values for 14 broad-leaved species from the eastern United States (Lutz and Chandler [10], Table 18) has been calculated. The most notable difference is the high nitrogen content of the Kade leaf litter. This appears to be a general effect, for it is confirmed by the high nitrogen content of the litter in the example from the Belgian Congo, and of freshly fallen leaves in two other areas of the Ghanaian forest (Nye [12]). The nitrogen content of the nature leaves in the tropical forest appears to be no greater than in temperate forest (Nye [12]), but the nitrogen in the temperate species must migrate to be branches to a much greater extent before leaf fall.

The contribution of the litter to the complete nutrient cycle is shown in Table 2.

TABLE 2

The nutrient cycle under mature forest
(lb. per acre per annum)

Addition to soil surface from forest	Wt. of material (oven dry)	Nutrient elements				
		N	P	K	Ca	Mg
Litter-fall	9,400	178	6.5	61	184	40
Timber-fall	ca. 10,000	32	2.6	5	73	7
Rain-wash		11	3.3	196	26	16
Total		221	12.4	262	283	63

2. Organic matter and nutrients in timber fall

The timber fall can only be roughly assessed, but even an approximate figure is useful for estimating its importance to the nutrient cycle. In a fully grown forest the annual timber fall will, on the average, equal the annual production of timber. This may be roughly estimated at 10,000 lb. per acre per annum. This is the average arrived at by Weck [16] for production of wood in tropical forest. At Kade, the standing timber represents an average production of 6,500 lb. per acre per annum over a 40-year period. If some allowance is made for timber that has fallen during this period, the estimate of 10,000 lb. is reasonable. The fall over a small area is, however, erratic, since it is greatly influenced by the fall of very large trees.

The composition of the falling timber will be similar to that of the standing dead wood analysed by Greenland and Kowal [3]

and shown in Table 1. This data is used to derive the estimate of annual nutrient addition to the soil as timber fall which appears in Table 2.

It will be seen that the falling dead wood may increase the rate of the calcium cycle by around one third, the phosphorus cycle by one quarter, the nitrogen cycle by one sixth, and the magnesium cycle by one eighth.

3. *Root decomposition and nutrient excretion from roots*

The contribution of root decomposition to the addition of organic matter cannot be measured directly, though a rough estimate is possible if the assumption is made that in a mature forest the productions of organic matter below and above the ground are in the same ratio as the weights of the roots and the aerial parts. In this forest Greenland and Kowal [3] found the weight of the roots to be 22,100 lb. per acre, and the weight of vegetation above ground 190,100 lb. per acre. These figures exclude material classified as stumps and dead wood. Since the annual production of litter and wood totals about 20,000 lb. per acre, the annual production of roots will be of the order of 2,300 lb. per acre. Since in this mature forest the net increase in the weight of roots present will be slight, this figure is also an estimate of the annual addition of dead root material to the soil.

A complete statement of the total return of nutrients to the soil has to take into account both the loss of nutrients from dying roots, and the excretion of nutrients from living roots into the soil. Examples of the latter are well known in crops approaching maturity, and may well occur seasonally among forest trees. It also seems likely that nutrients will be absorbed by the roots from spots at high potential in the soil and excreted to spots of low potential. The magnitude of these effects is unknown, though they are probably small compared with the total uptake and return of nutrients to the soil.

4. *The effects of rain wash*

The complete nutrient cycle. The monthly rainfall during 1959, its composition, and the nutrients added to the soil, both under forest and in the open, are shown in Table 3. The total amounts of nutrients leached from the leaves during the year are

TABLE 3

Composition of rainfall and amounts of nutrients falling in the open and under mature forest at Kade, Ghana — 1959

nth	Inches	Ppm						Lb. per acre					
		NO_3-N	NH_4-N	P	K	Ca	Mg	NO_3-N	NH_4-N	P	K	Ca	Mg
the open													
n.	1.24	0.5		0.04	2.3	3.8	1.1	0.1		0.01	0.6	1.1	0.3
b.	3.37	0.2		0.03	1.3	1.4	0.8	0.1		0.02	1.0	1.1	0.6
ar.	5.31	0.2	1.9	0.02	1.5	0.7	0.7	0.2	2.3	0.02	1.8	0.8	0.9
pr.	4.18	0.4	0.8	0.02	1.4	1.2	0.5	0.4	0.8	0.02	1.2	1.1	0.5
ay	12.97	0.0	0.3	0.02	0.8	0.6	0.4	0.1	0.9	0.06	2.4	1.8	1.1
ne	11.24	0.1	0.3	0.02	0.5	0.6	0.5	0.2	0.8	0.05	1.3	1.5	1.2
ly	10.93	0.1	0.5	0.01	1.0	0.2	0.7	0.2	1.2	0.02	2.5	0.5	1.8
ug.	2.05	0.1	1.2	0.02	0.6	0.6	0.6	0.0	0.6	0.01	0.3	0.3	0.3
ep.	4.14	0.1	1.0	0.01	1.1	0.4	0.5	0.1	0.9	0.01	1.0	0.4	0.4
ct.	5.81	0.2	0.6	0.03	0.9	0.4	1.2	0.3	0.8	0.04	1.2	0.5	1.6
ov.	8.73	0.1	0.8	0.04	0.6	0.6	0.6	0.2	1.6	0.08	1.2	1.2	1.2
ec.	2.77	0.4	0.6	0.04	1.7	1.6	0.2	0.3	0.4	0.03	1.1	1.0	0.2
otal	72.7							2.2	(10.3)	0.37	15.6	11.3	10.1
nder forest canopy													
an.	1.02	1.5		0.32	24.7	6.0	1.8	0.3		0.07	5.7	1.4	0.4
eb.	3.48	1.9		0.75	21.4	3.6	1.6	1.5		0.59	16.8	2.8	1.2
lar.	5.01	0.8	1.6	0.22	15.0	1.6	2.0	0.9	1.8	0.25	17.0	1.8	2.3
pr.	3.28	1.1	1.9	0.23	15.7	3.6	1.2	0.8	1.4	0.16	11.6	2.7	0.9
lay	11.36	0.4	0.8	0.27	15.8	1.8	1.7	1.0	2.1	0.69	40.5	4.6	4.4
une	9.76	0.4	0.6	0.17	10.0	2.6	1.4	0.8	1.4	0.37	22.0	5.8	3.2
uly	8.45	0.6	1.1	0.23	16.1	2.6	2.3	1.1	2.1	0.44	30.7	5.0	4.4
ug.	1.73	1.1	1.2	0.20	27.7	4.0	3.2	0.4	0.5	0.08	10.9	1.6	1.3
ep.	3.33	0.7	1.0	0.30	16.8	3.2	1.7	0.6	0.7	0.23	12.6	2.4	1.3
ct.	4.82	0.9	0.9	0.24	12.5	2.2	2.9	1.0	1.0	0.26	13.6	2.4	3.2
ov.	7.06	0.7	1.0	0.26	15.2	2.4	1.8	1.1	1.6	0.42	24.1	3.8	2.9
ec.	2.20	1.6	1.1	0.19	12.9	5.6	1.1	0.8	0.6	0.10	6.4	2.8	0.5
otal	61.5							10.4	(13.2)	3.66	211.9	37.1	26.0
utrients leached from canopy								8	3	3.3	196	26	16

calculated from this data, and the results are included in Table 2 to complete the data on the nutrient cycle. It will be seen that in comparison with amounts falling in the litter three times as much K, half as much P and Mg, and small amounts only of Ca, and N are leached from the leaves. The amounts of K leached are extraordinarily high. The extreme mobility of K is, however, in accord with Tamm's measurements [15]. In addition, the heavy canopy of leaves, which is maintained throughout the year, ensures that every drip collected has passed over a number of leaf surfaces.

The annual sum of the nutrients in the litter, timber fall, and

rain wash gives the rate of the nutrient cycle. If these totals are expressed as a percentage of the amounts of nutrients stored in the vegetation, as reported by Greenland and Kowal, the annual rate of turn-over of the nutrient capital is seen to be N–12, P–10, K–32, Ca–12, Mg–18 per cent.

Interception of rainfall by the canopy. At Kade during the year of collection 72.7 in. fell in the open, and 61.5 in. was collected under the forest. There was a total of 167 rain days. On the average therefore, the canopy intercepted 0.07 in. for each rain day.

The very inadequate data from the tropics on this subject has been reviewed by Mohr and Van Baren [11] (*l.c.* p. 48 and 96). They estimated that a dense virginal forest will hold up approximately 0.16 in. for each rain day.

In Fig. 2 the monthly rainfall under forest is plotted against the corresponding rainfall in the open. It will be seen that in spite of considerable variation in monthly rainfall, the canopy retained and evaporated 16 per cent of the rainfall in each month.

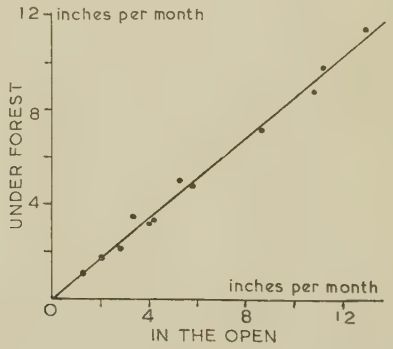

Fig. 2. Comparison of monthly rainfall in the open and under forest.

The possibility that significant amounts of rain flowed down the trunks of the trees was investigated. Each of the trunks of 12 trees was encircled with a strip of aluminium sheeting to form an upturned frill, whose base was sealed to the trunk with pitch. Water intercepted by the frill was led into a large container. During April and May less than 1 per cent of the rain falling (17 in.) flowed down

the trunks. Since its composition was similar to the leaf drips collection was discontinued.

Monthly additions of nutrients from rainfall and leaf wash. Records of the composition of rainfall in other regions, for example in Illinois (Larson and Hettick [8]), show that considerable variation from month to month is to be expected. Though the composition of the rainfall at Kade is likewise rather erratic, there is a general tendency for NO_3-N, P, K, and Ca, to occur in greater concentration during the dry months of December and January when the 'Harmattan' wind blows from the Sahara carrying dust in the air. It may be noted that during the short dry season in August when the wind is from the south-west and the air is clear, nutrient concentrations are the same as for the rainy months.

Analysis of the rainfall at these very high dilutions was subject to a number of positive errors. Blank determinations carried out on distilled water stored one month in the containing vessels indicated that the values for K, Ca, and Mg might be too high by about 0.3, 0.2, and 0.4 ppm. This would decrease the amounts falling in the year by 5, 3, and 6 pounds respectively. Since these errors are common to the analyses of rainfall collected under the forest the calculation of the amounts of nutrients leached from the leaves will not be affected.

The data in Table 2 show that the amounts leached from the leaves are closely related to the rainfall. There is only a slight tendency for each inch of rain to be less effective in wet than in dry months.

The distribution of nutrient concentration among the collecting vessels under forest was markedly asymmetrical, because of one or two notably high values. These were associated with more brown-coloured leachates and probably represented water that had been temporarily arrested by pockets of debris in the canopy. The irregularity in composition of the drips was such that the standard error for the average potassium concentration in any month was 28 per cent.

It will be noticed that insignificant amounts of NH_4-N, and only small amounts of NO_3 N are leached out of the leaves. This is of special interest to the problem of N-fixation under tropical forest. Ruinen [13] has suggested that nitrogen fixed by *Beijerinckia* sp. on the

leaves of the trees might offer an essential source of bound nitrogen, which might be taken up directly by the leaves or washed off by the rain. Beijerinckia has indeed been found on the leaves of the Kade forest by Meiklejohn (priv. comm.). The present data indicate, however, that little fixed nitrogen is being washed off the leaves by rain.

The relatively high concentrations of P and NO_3–N in February may be due to a flush of young leaf at that time.

Leaching of sodium, chloride, sulphate, and bicarbonate. In view of the large amounts of K leached from the leaves measurements were made to determine whether large amounts of other mobile ions such as Na, Cl, and SO_4 were leached; and also what anions accompanied the cations. The results of a more complete analysis that was carried out on the October and November rainfalls is given in Table 4.

TABLE 4

Composition of rainfall in the open and under forest canopy in October and November (milli-equiv. per litre)

Month	Cations					Anions					
	Na	K	Ca+Mg	NH_4	Total	HCO_3	Cl	NO_3	SO_4	H_2PO_4	Total
In the open											
Oct.	0.07	0.02	0.12	0.05	0.25	0.16	0.11	0.01	<.01	0.00	0.28
Nov.	0.04	0.02	0.08	0.05	0.19	0.12	0.09	0.02	<.01	0.00	0.23
Under forest canopy											
Oct.	0.09	0.31	0.35	0.06	0.81	0.50	0.16	0.07	<.01	0.01	0.74
Nov.	0.06	0.38	0.27	0.07	0.78	0.49	0.15	0.05	<.01	0.01	0.70

It is clear from this data that much the most important anion accompanying the cations leached from the leaves is bicarbonate.

The amounts of Na and Cl in the rainfall conform with values obtained in inland areas elsewhere (Eriksson [2]). During the two-month period in which 13.5 in. of rain fell it carried down the following amounts; Na – 3.9, Cl – 11.4, lb. per arcre. During the same period the amounts collected beneath the canopy were Na – 4.5, Cl – 14.7, lb. per acre. Thus only small quantities of these elements are leached from the leaves.

With regard to sulphate, Hesse [5], working in Kenya, noted an

increased concentration in rain drips under wattle and eucalyptus during the first rain after the dry season, but not during a period of three days when the rainy season was well advanced. During the two-month period at Kade less than 0,5 lb. per acre of sulphur was collected beneath the canopy.

SUMMARY

The amount and composition of litter and of rain, falling beneath a tropical high forest, have been measured. The litter fell continuously through the year. It was considerably richer in nitrogen than the litter of temperate forests. On the forest floor it decomposed extremely rapidly, at a rate of 1.3 per cent per day. Sixteen per cent of the annual rainfall was intercepted by the canopy and evaporated before it reached the ground.

Compared with amounts falling in the litter, very large amounts of K, significant amounts of P and Mg, but only small amounts of N and Ca were washed out of the canopy by the rain. The main anion accompanying the cations in the leaf drips was HCO_3. Little Na, Cl, or SO_4 was leached from the leaves.

The amounts of nutrients in the litter fall and in the rain wash, together with an estimate of the amounts in the timber fall, have been added together to give the rate of the nutrient cycle under this forest.

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A METHOD FOR ISOLATING FUNGI FROM SOIL MICROHABITATS

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INTRODUCTION

The initial aim of many studies on soil fungi is to obtain an accurate picture of the distribution of active forms in the many soil microhabitats. Many of the methods described for the isolation of fungi from soil do not allow such information to be obtained Warcup [9]. The hyphal isolation method Warcup [8] gives an accurate assessment of the active mycelial forms in soil, but it has certain disadvantages which have been discussed previously Warcup [9]; Parkinson and Kendrick [5]. In view of these disadvantages a washing technique has been developed in an attempt to provide a relatively rapid means of isolating active mycelial forms from soil.

Various washing techniques have been used for the study of fungi growing on root surfaces, and on fragments of organic material on, and in soil Simmonds [7]; Kurbis [3]; Glynne [2]; Chesters [1]; Robertson [6]; Harley and Waid [3], and it has been suggested Parkinson and Kendrick [5] that the use of washing techniques could be extended to the study of non-organic soil microhabitats. The technique to be described is a development of the techniques of Simmonds [7] and Chesters [1] and allows isolation of fungi from washed soil particles of various known sizes, the aim of the technique is to remove most or all of the fungal spores from the soil by vigorous, serial washings; thus any fungal isolates obtained from washed soil will probably be derived from hyphae attached to the soil particles, however the possibility that some isolates develop from chlamydospores or hypnospores cannot be excluded.

METHODS

a) *Apparatus*

The appartus used for the soil-washing experiments is shown in Figure 1. It consists of a perspex box (18 × 6 × 6 cm) with a removable front face. Into the box are fitted stainless steel sieves for the eventual separation of specific particle-size samples of the soil under experiment; the sieves fit into slots in the perspex sides of the box and are placed in descending order of mesh size from top to bottom in the box. In this work three sieve mesh sizes are used (1 mm, 0.75 mm, 0.2 mm). Perspex tubes are fitted to the top and bottom of the box: at the top of the box Tubes A and B are for the inflow of sterile water and maintenance of atmospheric pressure respectively; whilst at the bottom of the box Tube C allows the emptying of the box of washing water and Tube D allows the inflow of sterile air.

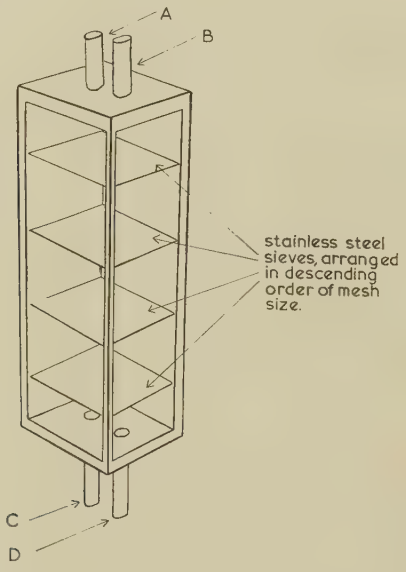

Fig. 1. Soil washing box (details given in text).

The box and tubing are sterilized by alcohol washing followed by sterile water washings. Soil is then introduced into the upper compartment of the box (*i.e.* it is placed on the sieve of largest mesh size). The front face of the box is placed in position and clamped to prevent subsequent leakage of washing water. Tube A is then connected to a reservoir of sterile water, Tube B fitted with an air filter, Tube C leads to a collecting container for washing water and Tube D was connected to an air filter and electric pump. All tubing, filters, *etc.* were sterilized prior to use in the apparatus.

b) *Washing technique*

Sterile water is run into the perspex box from the reservoir (150 ml was found to be a convenient volume of water for a box of this size), after this sterile air is pumped into the box producing vigorous agitation of the soil particles contained in the box. After the washing has continued for the desired time, the pump is stopped and the washing water drained out of the apparatus. This process is repeated as often as required.

Preliminary tests must be carried out to determine the most suitable quantities of soil and water which will allow maximum agitation and washing of the soil particles. Test experiments using a garden soil (Oxton, Cheshire) were performed to obtain information on the number of washings required by a single soil sample to remove all easily detachable fungal propagules from the soil particles. Soil samples were subjected to 45 washings (each of 2-min duration) with sterile water. The water from selected washings was collected in sterile centrifuge bottles, and each washing-water sample was then centrifuged at 500 rpm for 5 minutes to bring down small soil particles which has passed through the finest sieve (0.2 mm) in the washing box. One-ml samples of the supernatant water were then transferred to sterile petri dishes and incorporated with cooled, molten Czapek-Dox agar supplemented with yeast extract and adjusted to pH 5.0. Five dishes were prepared in this way for each washing-water sample. After the standard incubation period the numbers of fungal and bacterial colonies developing on each plate were recorded. This testing method is the same as that used by Harley and Waid [3], and the data obtained are similar to theirs. With increasing numbers of washings up to 30, there is a progressive decrease in the number of fungal propagules in the water samples plated. After the 30th washing little decrease occurs in the number of propagules washed from the soil sample.

Direct observations were made on the various washing water samples collected. Direct counts of fungal spores and mycelial fragments were made (5 subsamples were taken from each washing-water sample, and 50 observations made on each subsample, *i.e.* 250 observations were made for each washing-water sample). The results of these observations show similar decreases in the amounts of spore and mycelial material in the washing water with increasing numbers of washings to those shown by the plating of water samples.

RESULTS

The results of these tests are given in Table 1 and Figures 2 and 3. The number of washings required for efficient removal of spores will vary from soil to soil, but for the soil under experiment the method appears to be efficient in removing most of the spores if 25 to 30 washings are used. As would be expected some mycelium is also washed out, but in relatively small amounts.

In the technique described, all soil particles smaller than 0.2 mm diameter pass out of the box when washing water is drained off.

Such soil may be collected and washed by repeated centrifugation. In preliminary experiments with such small particles it has been found convenient to separate them into two classes: — 1. Those particles brought down by centrifuging at 500 rpm (*i.e.* RCF of 65 × g) for 5 minutes; 2. Those particles brought down by centrifuging at 1,000 rpm (*i.e.* RCF of 260 × g) for 5 minutes. It was found that 30 changes of water (sterile) was the minimum to allow efficient washing of these smaller particles in the soil under experiment. By comparison with the use of the washing apparatus

TABLE 1

Results from the plating and direct observation of washing water from washing box

No. of washings	Plating		Direct observation	
	Av. No. fungal colonies	Av. No. bact colonies	Percentage freq. of occurrence, spores	Percentage freq. of occurrence, mycelium
1	30.0	20.0	30.4	6.4
3	8.2	23.2	17.0	4.5
6	3.6	4.6	12.0	3.6
10	6.8	4.6	—	—
12	6.4	2.0	—	—
16	3.2	2.0	7.4	3.2
20	2.0	2.0	7.6	0.4
25	1.2	2.0	7.2	0.8
30	0.0	4.0	6.6	0.6
35	0.3	4.6	—	—
45	0.6	3.6	5.3	0.6

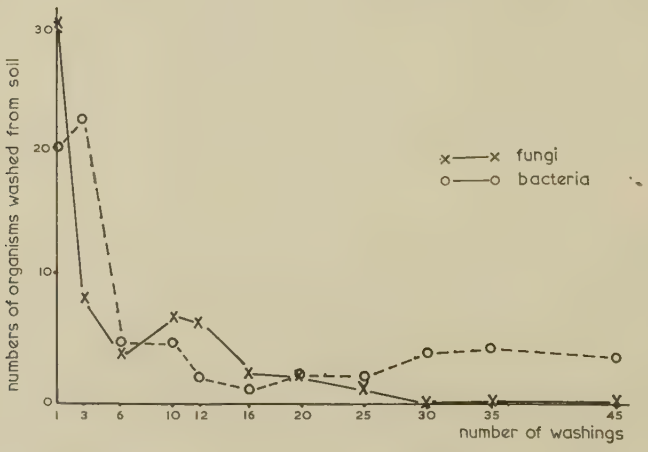

Fig. 2. Numbers of fungi and bacteria removed from soil by serial washings with sterile water (as estimated by the plating technique).

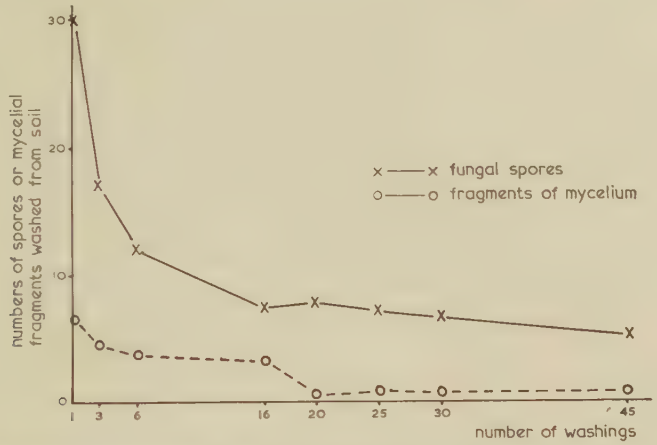

Fig. 3. Numbers of fungal spores and mycelial fragments removed from soil by serial washings with sterile water (as estimated by the direct observation technique).

described above, the separation and washing of soil particles smaller than 0.2 mm is much more time consuming. Also the technique is not so efficient in removing fungal spores.

The technique described was applied to an investigation of the active fungi in a garden soil (Oxton, Cheshire). Details of this soil are given in Table 2. Soil samples were taken from a depth of 2 to 6 inches and from these samples isolation of fungi was effected by several techniques: Dilution plates, Warcup soil plates, and the plating of washed soil particles. In all these techniques the isolating medium used was Czapek-Dox + yeast extract agar adjusted to pH 5.0.

TABLE 2

Analytical details of the soil under investigation	
pH	6.2
Organic matter . .	8.5%
Gravel	9.5%
Coarse sand. . . .	54.5%
Fine sand	19.5%
Silt	5.3%
Clay	5.3%

Table 3 gives the results of these experiments, the results being expressed as the percentage of the *plates* colonized by the various fungi. From these data certain interesting points emerge: —

(i) Heavily sporing species such as *Penicillium* spp., *Aspergillus* spp., and *Trichoderma viride* which were isolated with a high percentage frequency from unwashed soil, were isolated much less frequently from washed soil. The conclusion that such species are present in the soil mainly as spores is supported by the fact that they were also isolated with high frequency on dilution plates prepared from the water used for the soil washing.

TABLE 3

Comparison of the frequency of occurrence of fungi (recorded as percentage of plates colonized) in washed and unwashed soil

Species isolated	Soil plates of unwashed soil	Dilution plates from washing water	Soil plates from four fractions of washed soil			
			Fraction I (> 1 mm)	Fraction II (0.75-1.0 mm)	Fraction III (0.2-0.75 mm)	Orga fragm (1-2 m
Absidia sp.	5	—	—	—	—	—
Mortierella sp.	35	30	10	10	—	—
Mucor hiemalis	90	40	—	—	20	40
Zygorrhynchus moelleri	15	15	—	—	—	—
Aspergillus niger	55	35	20	10	—	—
Aspergillus versicolor	95	100	20	20	30	10
Cephalosporium sp.	—	5	—	—	—	—
Cladosporium herbarum	5	—	—	—	—	10
Coniothyrium sp.	—	—	—	—	—	20
Curvularia sp.	—	—	—	10	—	—
Cylindrocarpon radicicola	5	5	10	—	10	50
Fusarium spp.	100	40	100	90	90	100
Gliocladium spp.	5	15	—	—	—	—
Memnoniella sp.	25	—	—	—	—	—
Monotospora sp.	35	5	20	20	—	—
Penicillium spp.	45	60	—	30	20	—
Sporotrichum sp.	—	—	30	—	40	—
Stemphylium sp.	10	—	10	10	—	—
Stysanus sp.	—	5	—	—	—	—
Torula sp.	—	—	—	—	—	20
Trichoderma viride	85	55	30	30	20	—
Sterile white forms	20	10	60	50	50	60
Sterile dark forms	5	15	—	—	20	40
No. species isolated	17	15	10	10	9	

(ii) Certain fungi *e.g.* sterile forms, *Sporotrichum* sp., *Curvularia* sp., which were absent from, or present with low frequency in,

unwashed-soil isolates, were more frequently isolated from washed soil particles.

(iii) *Fusarium* spp. had a high percentage frequency of occurrence on the plates prepared from both washed and unwashed soil particles.

(iv) With the preliminary data provided here it is unwise to consider any possible localization of fungal species in the washed microhabitats plated (*i.e.* mineral soil particles of different sizes and organic fragments). However it may be seen (Tables 3 and 4) that *Cylindrocarpon radicicola* is present with the greatest frequency on organic fragments.

DISCUSSION

The data provided are similar to those presented for another Cheshire soil (Parkinson and Kendrick [5]), and support the contention that the plating of washed substrates yields a large percentage of isolates which are mycelial in origin. The tests employed (plating of the washing water *etc.*) show the efficiency of this method in removing spores from soil. Another important advantage of this technique is the speed with which the required number of serial washings (usually 20 to 30) can be accomplished.

The plating of discrete soil particles (both washed and unwashed) allows a comparison to be made of fungi isolated from the two types of substrate (washed and unwashed) using the percentage of the particles colonized as the basis of this comparison. Table 4 gives data in which fungal occurrence is recorded as a percentage of the total number of particles plated. In general these data support those presented in Table 3. Recording fungal occurrence as a percentage of the number of plated particles gives a more accurate picture of the abundance of these forms, but this method of recording data is only feasible when discrete single soil particles can be plated.

The value of this technique has been increased by the use of smaller washing boxes (13 × 4 × 4 cm), hence less sterile water is used for the serial washings. The apparatus now in use consists of a number of the smaller boxes mounted in such a fashion as to receive sterile water from a common reservoir. Thus several soil samples may be washed concurrently with almost the same speed as that

TABLE 4

Comparison of frequency of occurrence of fungi (recorded as percentage of plated soil particles colonized) in washed and unwashed soil

Species isolated	Unwashed soil particles	Washed soil particles			
		>1 mm	0.75–1.0 mm	0.25–0.75 mm	Organic fragments
Mortierella sp.	—	1	1	—	7
Mucor spp.	14	10	2	—	12
Aspergillus spp.	34	4	4	4	1.5
Cladosporium herbarum	—	—	—	—	4
Curvularia sp.	—	—	—	4	—
Cylindrocarpon radicicola	—	—	—	5	10
Fusarium spp.	46	41	20	21	52
Monotospora sp.	7	4	3	—	—
Penicillium spp.	15	—	4	4	—
Sporotrichum sp.	—	4	—	6	—
Stemphylium sp.	—	1	1	—	—
Torula sp.	—	—	—	1	1.5
Trichoderma viride	34	5	5	1	—
Sterile white forms	2	9	13	14	9
Sterile dark forms	—	—	—	5	10
No. species isolated	7	9	9	10	9

for one soil sample. This apparatus is being used for the detailed study of various Cheshire soils.

SUMMARY

A technique is described for the separation and washing of soil microhabitats (the separation being on a size basis). Tests of the efficiency of the washing technique show that, for the soil under experiment, 25 to 30 washings are required to remove most of the fungal spores; the number of washings required will vary from soil to soil. Data is given on the use of this method for fungal isolations from a cultivated soil.

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ECOLOGICAL STUDIES ON *TAMARIX APHYLLA* (L.) KARST.

III. THE SALT ECONOMY

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INTRODUCTION

One of the most interesting phenomena characterizing the Tamaricaceae family is the existence of an "active salt economy". *Tamarix aphylla* is not outstanding in this respect (Waisel [13 14]). Salts absorbed by the roots in the various soil layers are excreted on to the surface of the leaves by special salt-excreting glands, thus completing a continuous salt movement through the plant body. The salt crystals cover mainly the adult branches of the trees. The excretion is most easily detectable during the dry summer months, a season in which the salt crystals are not washed off, remain on the branches, and give the plant its characteristic grey appearance.

It was assumed that *T. aphylla* is able to overcome salt concentrations injurious to the various tissues, by excretion of the salt excess. This tree may therefore be able to grow in saline habitats though in fact, unlike other Tamarix species, it does not occur there naturally. Being one of the very few large trees in the genus Tamarix, *T. aphylla* could play an important part in the afforestation of saline habitats. Therefore, an investigation into the salt tolerance and exudation of *T. aphylla* was undertaken.

PART A. SALT TOLERANCE

Halophytes are able to remain alive for long periods in high salt concentrations, with little or no additional growth (Hayward and

Wadleigh [5], sometimes followed by the dying out of other tissues. In contrast to this kind of salt tolerance, the salt tolerance of a crop or a forest plant should be measured only by the rate of increase in its "growing material". In the following experiment the growth of *T. aphylla* in NaCl solutions of various concentrations was investigated.

Material and methods

A large number of cuttings (10 cm long and 1 cm in diameter) were planted in pots filled with sand free per pot. During the first two weeks the cuttings were watered with tap water only in order to exclude the effect of the salt solution on the rooting and sprouting of the cuttings (Waisel [12]). After this period the cuttings were transferred to saline irrigation. The pots were irrigated three times a day during the rest of the experiment with NaCl solutions of the concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 *M*. The frequent irrigations permitted the various salt concentrations in the pots to be maintained at a constant level. Every second day, the plants were given an additional irrigation with a nutrient solution (Hayward *et al.*[4]) in order to provide an undisturbed mineral nutrition.

At 13, 28, 62 and 81 days after planting the shoots from five pots were harvested, dried in an oven at 80° C and weighed. The results are given in Fig. 1.

Results and discussion

Figure 1 shows a remarkable decrease in the growth intensity with increase in the concentration of the NaCl irrigation solution.

Under irrigation with 0.1*M* and 0.2*M* NaCl solutions, growth of the saplings was considerably depressed as compared with the growth of the controls. Actually growth had already ceased at the concentration of 0.3*M* NaCl, but the plants remained alive up to the concentration of 0.5 to 0.7*M*, as determined by the tetrazolium viability test (Parker [9]). The test was conducted on slices taken about 3 cm from the base of the stem. At 0.5*M*, *Tamarix aphylla* frequently exhibited some growth of new shoots, but the intensity of this growth was so low that it was not sufficient to compensate the loss of dead tissues, and a decrease in weight was observed. It is worth noticing that this concentration is very near to the concentration of sea water.

From the above results it may be seen that *T. aphylla* is only moderately salt tolerant.

The slow growth rate exhibited in the 0.1*M* and 0.2*M* NaCl

might nevertheless still result in large trees, although only after a very long time. However, at this level of salinity the plant will be very sensitive to change in the moisture content of the soil, and a short period of drought might cause serious damage to the trees,

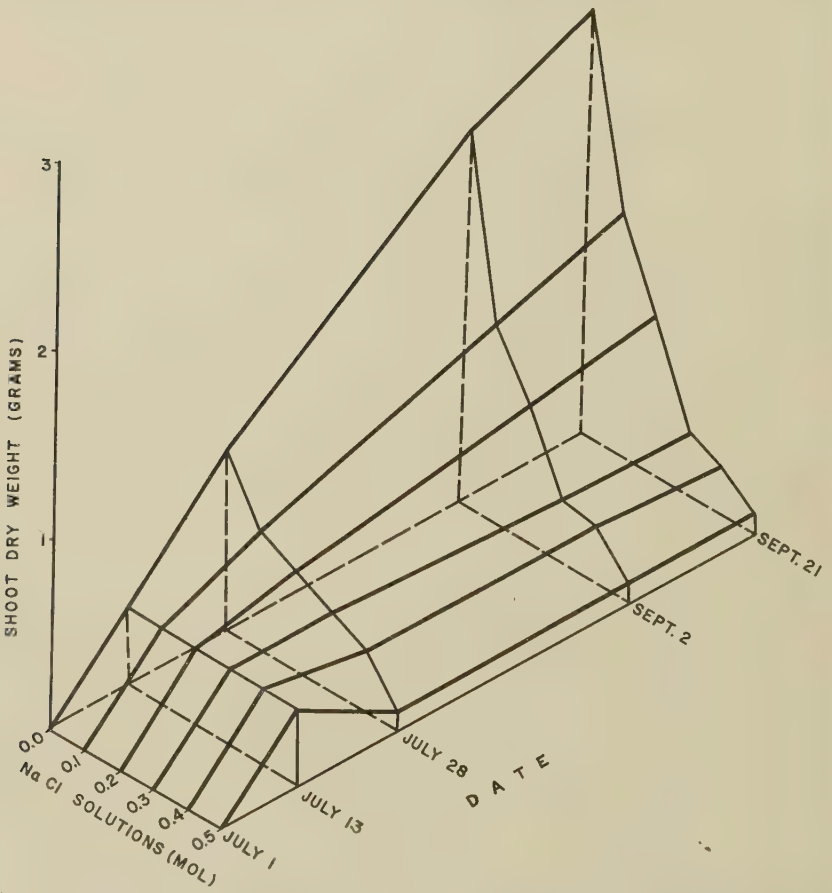

Fig. 1. Three-dimensional growth curve of *T. aphylla* saplings under irrigation with various NaCl solutions.

as has frequently been observed in other plants growing in similar habitats. Afforestation of saline soils with *T. aphylla* will, therefore, be hazardous.

In Israel, salines are not included among the natural habitats of *T. aphylla*, as their salt concentrations are far higher than that the trees tolerate. However, in various places *T. aphylla* trees are

found along water courses, in and on saline borders, and in a few cases even very close to the coast of the Dead Sea. Our opinion is that in these cases, *T. aphylla* enjoys an underground flow of non-saline water, although, as will be seen later, a part of its roots might be immersed in saline water. Such explanation might suit the records of Muschler [7] in Egypt, who counts salines among the natural habitats of this tree.

PART B. SALT EXCRETION

The salts excreted by the leaves of *Tamarix aphylla* seem to have both direct and indirect effects on the life and behaviour of this tree. The reader is referred to the papers of Volkens [12], Arisz *et al.*[1] and Helder [6].

Salt excretion by *T. aphylla* trees takes place on non-saline soils as well as on relatively saline ones, but the salt differs in its composition. On the shoots of young trees planted on non-saline sand dunes, the roots of which do not attain a great depth, white tasteless crystals (largely $CaCO_3$ – see below) accumulate. On the contrary, adult trees in the same region, the roots of which penetrate into the saline loess below the dunes, excrete very salty crystals (mainly NaCl). This implies that the salt composition and content of the soil in the root zone might be reflected in the salt crystals excreted by the shoots of *T. aphylla.*

The following experiments were conducted in order to study both quantitatively and qualitatively the salt excretion of *T. aphylla* trees, under natural field conditions as well as under controlled soil salinity.

Material and methods

Cuttings of *Tamarix aphylla* were planted, 9 per pot, in large pots filled with well-washed sand. The plants were grown in a greenhouse in order to avoid accumulation of dust on the plant twigs. The experiment was conducted in three replications.

During the first two weeks after planting, the cuttings, which were watered with tap water, took root and developed a few branches each. Later on, the branches were thoroughly shaken in distilled water, in order to wash all the existing salt particles from the shoots. During the following two weeks the plants were given various irrigation treatments with NaCl, KCl, $CaCl_2$, and $MgCl_2$ solutions. Controls were irrigated with tap water. At the end of this period, the plant shoots were harvested, and thoroughly

washed in a measured amount of distilled water, about pH 6. The resulting solution was analysed. Chlorine was titrated with $AgNO_3$, and the cation content was determined with a flame photometer. Results are given in Table 1.

Concomitant qualitative determinations of the excreted salts by the plants under various irrigation treatments and in their natural stands are summarized in Table 2. The iron and bromine determinations were made according to the methods brought by Feigl [3].

Results and discussion

The effect of the nature of the irrigation solution on the salt excretion can be seen in Table 1.

TABLE 1

Salt excretion by *Tamarix aphylla*. Quantitative analysis

Irrigation solution (diluted in tap water)	Salt excretion in mg per 100 g fresh plant material			
	Ca	K	Na	Cl
Control (tap water)	43.8	17.6	262.7	54.9
0.1 *M* NaCl	46.1	17.1	461.6	126.2
0.2 *M* ,,	40.9	16.7	754.3	215.2
0.3 *M* ,,	42.3	17.1	669.0	128.3
0.4 *M* ,,	39.6	14.6	401.3	130.7
0.1 *M* KCl	41.8	23.1	155.0	191.0
0.1 *M* $MgCl_2$	42.6	9.0	158.0	106.0
0.1 *M* $CaCl_2$	44.7	15.0	113.7	56.5
0.2 *M* ,,	40.0	15.3	77.9	52.5
0.3 *M* ,,	42.3	13.0	71.6	55.7
LSD .... $P = 0.01$	NS*	3.68	49.70	24.79
LSD .... $P = 0.05$		2.68	36.24	18.08

* No significant difference between treatments

As compared to the control, a very remarkable increase in the amount of chlorine excreted is exhibited by the plants which were irrigated with NaCl, KCl and even $MgCl_2$ solutions. On the other hand, *T. aphylla* saplings which were irrigated with $CaCl_2$ solutions of three concentrations, excreted chlorine in amounts equal to those excreted by the control plants.

The amounts of calcium excreted by the plants under all the treatments, including those irrigated with $CaCl_2$ solutions, did not show significant differences. Thus, a well-organized mechanism seems to exist and to control the calcium excretion which is independent of the composition of the outer solution. Furthermore,

interpretation of the results presented by Oppenheimer and Halfon-Meiri [8], shows that this mechanism seems to be independent even of the factors controlling calcium retention in the leaves.

The sodium excretion was quantitatively the highest of all the three cations examined. An optimum curve was found to relate the amount of sodium excreted to the concentration of NaCl in the irrigation solution, with a peak at 0.2*M* NaCl. in the case of the 0.4*M* treatment, the solution caused reduced growth, possibly due to osmotic or toxic effects which may have affected some essential mechanism of excretion. This may have led to the observed decrease not only in sodium excretion, but in chlorine and potassium excretion as well.

The presence of potassium, magnesium and calcium ions in the irrigation solutions depresses the excretion of sodium, as compared with the controls irrigated with tap water. In the case of $CaCl_2$, which was applied in three concentrations, this decrease was positively correlated with the concentrations of $CaCl_2$ in the irrigation solution.

Potassium was excreted in equal amounts by the control plants and by those treated with the three lower concentrations of NaCl. Thus, unless not at toxic concentrations, an excess of sodium in the external solution did not depress the amounts of potassium excreted. On the other hand, as mentioned above, an excess of potassium in the irrigation solution caused a decrease in the amounts of sodium excreted.

The problem of the interrelations between potassium and sodium was lately discussed by Sutcliffe [11] and Bange [2], and the reader is referred for theoretical explanation to these publications.

It is of course of interest to note here the parallelism in behaviour between absorption of these alkali ions (Reifenberg and Rosovsky [10]), and their excretion.

Plants irrigated with $CaCl_2$ and $MgCl_2$ solutions exhibited a decrease in the amount of potassium excreted. This effect is more noticeable in the case of $MgCl_2$ treatments, where the magnesium ions probably interact not only as interfering ions, but exert their relatively high toxicity. As seen above, toxic concentrations of sodium have a similar effect.

Excretion of nitrate, phosphate and sulphate took place when-

ever the plants were supplied with sufficient amounts of these ions. No excretion of iron and a rare excretion of magnesium were observed.

TABLE 2

Salt excretion by *Tamarix aphylla*.
Qualitative analysis.

Irrigation solution (diluted in tap water) or environment	Ions tested	
	Present	Absent
Under experimental conditions		
Control (tap water)	Calcium, chlorine, potassium, sodium	Sulphate
$FeCl_3$ 1%	Chlorine	Iron
KNO_3 1%	Nitrate, potassium	
$Ca(NO_3)_2$ 1%	Calcium, nitrate	
K_2SO_4 1%	Potassium, sulphate	
$MgSO_4$ 1%	Sulphate, magnesium (traces)	
K_2HPO_4 1%	Phosphate, potassium	
$CaHPO_4$ 1%	Calcium, phosphate	
Under field conditions		
Young saplings on deep sand dunes	Calcium, carbonate, chlorine, potassium, sodium	Sulphate
Adult trees on sand dunes underlaid by saline loess	Calcium, chlorine, potassium, sodium	
Trees 50 metres from the Dead Sea	Bromine, calcium, chlorine, potassium, sodium	
Trees 200 metres from the Dead Sea	Calcium, chlorine, potassium, sodium	Bromine

Semiquantitative analysis of the salts excreted by *T. aphylla* plants in some of their various natural habitats shows that the plants growing on non-saline soils excrete large amounts of calcium carbonate, whereas those reaching saline soil layers excrete mainly sodium chloride. This is still more pronounced by other Tamarix species growing under similar habitat conditions.

An interesting result was obtained while testing the salt crystals excreted by Tamarix trees near the Dead-Sea coast. These crystals were found to contain bromine. As the water courses which provide the main source of water for these plants contain almost no bromine, we must assume that either a soil layer saturated with this mineral exists near the coast or that a part of the roots come into contact with very dilute water of the Dead Sea. The trees which exhibited bromine excretion were found scattered in a belt extending to about 100 metres from the coast of the Sea.

From all the above results it may be concluded that *Tamarix aphylla* can be to some extent an indicator of the soil salinity in general and of the composition of its salts. This may in many instances save much time and labour in agricultural survey.

SUMMARY

Young saplings of *Tamarix aphylla* were investigated for their growth intensity under saline irrigation, as well as for the lethal limits of salinity they can tolerate. The saplings were found to be quite sensitive to the addition of NaCl to the irrigation solutions and died when the concentration of the solutions was raised to about 0.7*M* NaCl. Growth was depressed even by irrigation with a 0.1*M* NaCl solution, and no increase in the plant weight was obtained under irrigation with a 0.3*M* NaCl solution. It, thus, appears that *T. aphylla* is not suitable for afforestation of saline habitats.

Some aspects of the salt excretion of *T. aphylla* were investigated. Sodium and chlorine were found to be excreted most readily and exhibit an optimum type of curve, when the excretion is plotted against the concentration of the irrigation solution. Interrelations between the various ions in the irrigation solutions and the amounts of salts excreted, were found to take place and to parallel the behaviour of these ions in their uptake by the roots.

T. aphylla seems to be able to reflect the soil's salinity and the composition of its salts, by the excreted salt crystals.

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STUDIES ON CATION-EXCHANGE CAPACITIES OF ROOTS

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INTRODUCTION

Although no fully evidenced concept of the mechanism whereby ions enter plant roots has been advanced, it is generally held that several processes are involved. Of these diffusion, exchange—adsorption and ion-binding compounds have been given most attention. Since Jenny and Overstreet [20] and Lundegårdh [22] displaced the prevailing idea of exuded CO_2 as the main agency whereby cation intake by roots took place, the concept of adsorption at root surfaces by exchange reactions suggested by these and other authors [20 22 7 23] has been accepted as one possible mechanism. However, although the existence of exchange capacity properties of living and dead plant tissues in general was demonstrated by Devaux [5] as early as 1916, the degree of importance of such a property in adsorption processes still awaits conclusive confirmation. As a working hypothesis for plant physiologists and soil chemists to explain how various plant species survive on soils with cations present at a low level of availability [12], and as a possible explanation of a differential uptake of mono- and divalent cations (*e.g.* [17 27 28 31 35]), the exchange—adsorption concept has stimulated much work on the cation-exchange capacities of plant roots.

Different methods for estimating root-exchange capacities have rarely been compared, and so it is difficult to know the compatibility of results by workers using different methods. In the work now to be described CEC values obtained in several ways were compared, and factors affecting the various estimations were examined. The majority of published data refer to very young

plants grown in a greenhouse. The purpose of this work was to compare CEC values of species grown under varying environmental conditions, to estimate variations of CEC within a species and to assess the validity of comparison between species. An attempt was also made to characterise the acidic groups responsible for the negative charges on root surfaces and to investigate the relationship between CEC values, and relative uptakes of mono- and divalent cations by plant species grown under controlled nutrient conditions.

MATERIALS AND GENERAL REMARKS ON METHODS

In all estimations, unless otherwise stated, fresh root material was used. Roots were washed in demineralised water and lightly dried by a short spin in a basket centrifuge. Where possible whole root systems were used. When very old, woody roots were used, fine and coarse roots were compared separately. In the greenhouse, plants were grown in flint, with dilute Hoagland solution ($^1/_5$ full strength) with additional micronutrients. Pots were occasionally leached with distilled water and fresh nutrients added. Samples for CEC and other estimations were taken from two pots and analysed separately. It is realised that metabolic activity may effect ion uptake, but no attempt was made to restrict such activity by doing reactions at low temperature. When roots were in contact with salt solutions for several hours they were kept at 5°C. The inadequacy of the dry weight as reference basis has been stressed by Smith and Wallace [33], but there is no satisfactory method for estimating active root surfaces and therefore the dry-matter basis was used. CEC is expressed as me per 100 g dry matter (24 hours at 70°C).

PART I

COMPARISON BETWEEN VARIOUS METHODS FOR ESTIMATING CEC VALUES AND BUFFER CURVES OF PLANT ROOTS

CEC values have in general been estimated by one or other of the following three methods:— estimating exchange acidity of roots, exchange reactions between roots and salt solutions, and by measuring cation activities of roots with a clay-membrane electrode technique. In the present work the two first methods were used and also an isotopic-exchange method using Ca^{45}.

Estimation of CEC values by titration of roots saturated with H^+ ions

In estimating CEC values by titration of liberated H^+ ions, the

roots have generally been hydrogen-ion saturated by electrodialysis. This is laborious and may have other effects on roots than removing cations. Some workers [4 36] have therefore saturated roots with H^+ ions by briefly immersing them in acids. In preliminary experiments here, solutions of HCl in *N* KCl (pH 2 or 3) were left with root material for several hours and suspensions subsequently titrated with KOH. The root/solution ratio was 1 : 10 and the time taken to neutralise the suspensions was 5 to 10 minutes. The end point was defined as the amount of KOH necessary to maintain a pH of 7.0 to 7.1 for one minute. CEC values were obtained from difference in titre between solutions with and without roots. While CEC values calculated for pH ranges 2 to 7 or 3 to 7 varied for different species, they tended to become similar in the pH region 5 to 7. Thus pea and oat roots gave CEC values of 50 and 23 for the pH range 2 to 7, and 8 and 9 respectively for the less acid range (5 to 7). CEC values calculated for pH range 2 to 7 were greater when roots were acidified with *N* KCl and 0.1 *N* HCl then with *N* KCl and 0.01 *N* HCl. Thus grass roots yielded values of 28 and 15 for the two concentrations respectively. The above method was modified to ensure that neutralisations of root suspensions were compared with those of solutions in equilibrium with the roots. Roots were left in stoppered test tubes for 15 hours at 5°C with sufficient HCl in *N* KCl to give a suspension at pH 2. Root/solution ratio was 1 : 15. The pH of the suspension was checked next day and half the added volume pipetted off as clear solution. This and the remaining root suspension were separately titrated rapidly to pH 7.0 to 7.1, the end point defined as the amount of KOH necessary to maintain pH 7.0 to 7.1 for one minute. CEC was calculated from the difference in titres, representing the amount of base adsorbed by the root material in equilibrium with a solution of the same pH. The value of this method was somewhat lessened by the drift in acid direction from pH 6 and upwards, exhibited by the root suspensions but not by the clear supernatant solutions. Drake *et al.* [6] also found a similar drift and recommended an arbitrary time limit of the titration. This rapid drift has been attributed to various causes; such as reaction taking place with colloidal material inside the roots, or the presence of acids of varying strength or changes in the intake of ions with time [6]. Holden [14] observed an acidic drift when minced leaf suspensions were neutralised because of enzymic

breakdown of pectin to pectic acid and methanol. In collaboration with Holden, methanol was found when water or acid root suspensions were titrated, and the presence of pectin esterase was also verified. Further evidence that pectin causes the rapid acid drift comes from the fact that there was no drift and CEC values were lower, when roots were pretreated with commercial pectinase, ammonium oxalate or boiling water. The last treatment inhibited pectin esterase and simultaneously dissolved labile active acid groups. Besides the rapid K sorption during neutralisation of hydrogen saturated roots, an increase in CEC values over several days was found when daily neutralised root suspensions were left at 5°C. A root suspension with CEC values 6.5 and 10.0, 1 and 15 minutes after reaching pH 7.0 on the first day of neutralisation, was left and again neutralised on the following and subsequent days. The pH values of the suspension were 4.3, 5.9, 6.5, and 6.8, on the 2nd, 3rd, 4th, and 5th day after first neutralisation while corresponding CEC values were 17.9, 24.5, 27.4, and 28.9.

Mouat and Walker [29] also found increases in CEC values during the first 2 hours and ascribed the sorption after the first 30 minutes to possible metabolic absorption. No explanation can be advanced for these results in the absence of a simultaneous estimation of metabolic activity, but they illustrate the difficulties involved in defining CEC values by this method. Table 4 compares CEC values by this method and those by other methods.

Buffer curves of plant roots

If exchange reactions are an integral part of cation uptake by roots, the origin of the negative charges on root membranes is of interest. Pectin is a constituent of root cell walls. The lowering of CEC values by removal of pectin from roots, taken together with the general relationship between pectin content and CEC values found by Keller and Deuel [21], suggests that the carboxylic groups of pectin may be some of the main acidic groups.

Figure 1 represents the dissociation curves of some root suspensions, obtained by the titration technique outlined above. The ordinate represents pH values of root suspensions before titrations. The distance of each point from the right hand edge of the graph (*i.e.* the value $1 - \alpha$ in the Henderson equation for the dissociation of weak monobasic acids) was obtained by dividing the measured

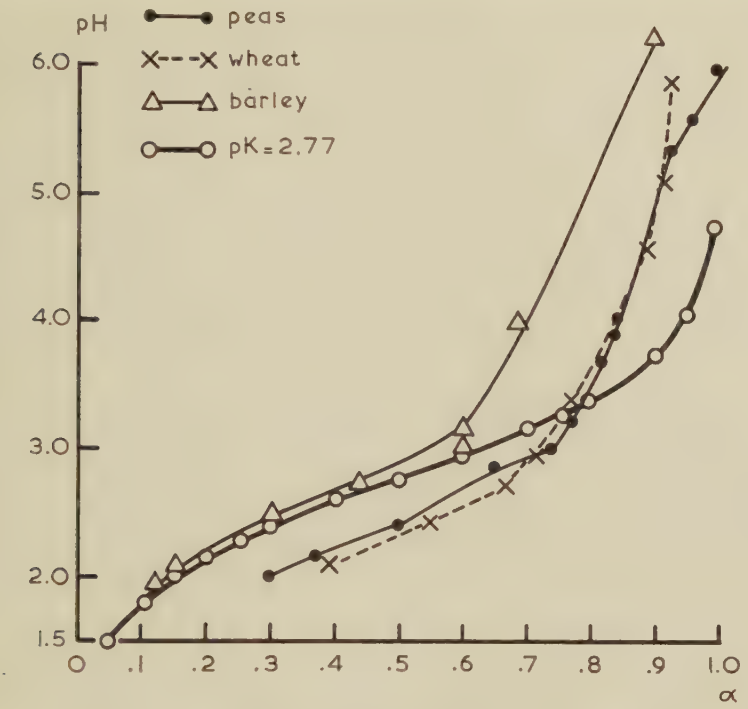

Fig. 1. Dissociation curves of different root suspensions.

difference in titre between supernatant liquid and remaining root suspension by the limiting difference for the lowest pH value used. This limiting value represented a measure of total hydrion dissociation of the roots at pH 7.0. For comparison, the theoretical dissociation curve pK 2.77 is also shown. Although the curves of roots refer to concentration and not to activities, as in the pK 2.77 curve, this would not greatly affect the differences. The dissociation curves of roots suspensions of young wheat and pea plants fall considerably below the 2.77 pK curve for pH values below 3.5, while departures are above for higher pHs. As a contrast the curve of barley roots conforms almost to the Henderson curve up to pH 3, whereas points above this pH depart in the same way as those of pea and wheat roots. Sarek and Schofield [30] found similar departures from the Henderson curve with pectic acid preparations. No final explanation can be offered for this behaviour. The possibility exists that labile acidic groups could be liberated with pro-

gressive neutralisation. Bonner [3] showed an increase in the pK value of pectic acid from 2.77 to 4.18 in the pH range 2.43 to 5.37 caused by unknown factors. Sarek and Schofield [30] suggested a "bridge" formation between a hydrogen of one —COOH group and an oxygen of another, either —COOH group a $—COO^-$ or group, to account for the behaviour of pectin. The absence of any clearly defined inflexion points of the curves prevents calculation of the pK of the root suspensions, but the results are consistent with the view that a least part of the acidic groups are carboxylic groups derived from pectic compounds.

CEC estimations by exchange reactions with various cations

Roots were saturated with barium or potassium by immersion in chloride solutions. The loosely adhering cations were removed by two rinses in distilled water, and the adsorbed Ba^{++} or K^+ displaced by sulphuric acid and ammonium acetate respectively of the same concentration and for the same time as in the chloride solutions.

TABLE 1

The effects on CEC values of varying root/solution ratio, concentration of salt solution, time of contact between roots and salt solution and time of immersion of roots in water before displacement of adsorbed cations

Salt solution	Plant species	CEC values			
		Root/solution ratio			
		1/10		1/20	
KCl (1.0*N*)	Barley	28.1		40.0	
$BaCl_2$ (0.01*N*)	Peas	46.5		50.0	
,, (0.01*N*)	Ryegrass	23.4		24.8	
		Concentration of cation			
		1.0*N*	0.1*N*	0.01*N*	0.001*N*
KCl	Wheat	14.5	3.7	2.0	0.9
$BaCl_2$	Wheat	13.5	14.0	—	12.0
		Time (min) in salt solution			
		1	5	10	15
KCl (1.0*N*)	Peas	38.9	80.5	100.4	115
$BaCl_2$ (0.01*N*)	Peas	40.1	39.0	40.5	39.0
		Time (min) in second wash			
		0.5	1	5	30
KCl (1.0*N*)	Peas	39.2	30.0	13.0	5.1
$BaCl_2$ (0.01*N*)	Wheat	13.8	—	13.8	14.0

Ba was estimated by titration and K by flame photometer. The following factors were varied: 1) root solution ratio 2) concentration of cations 3) duration in salt solutions, and 4) duration of second wash, following 30 seconds in first water.

The results in Table 1 show that CEC values estimated by exchange reactions with $BaCl_2$ are not affected to any great extent either by duration of contact or by the concentration of Ba^{++} but small increases occurred when root solution ratio was increased. In contrast, CEC values measured by adsorbtion of potassium instead of barium by the roots depended greatly on experimental conditions. Thus prolonged immersion of the roots in water lowered CEC values substantially. This effect seems to imply that these ions diffused reversibly, or that the K^+ ions were bound so lightly that they dissociated in water, as suggested by Epstein and Legett [10]. Comparison between CEC values obtained with the two cations showed that, at concentrations below normality, roots possessed greater affinity for Ba^{++} than for K^+. This agrees with results of other workers [1 28 29] who compared K^+ and Ca^{++} values. When estimating CEC by adsorption of K^+ or Ba^{++} ions, root material was placed for one minute in N and 0.01 N potassium — or barium chloride solution — subsequently washed in two waters for 30 and 60 seconds, and the adsorbed cation displaced by N ammonium acetate or 0.01 N sulphuric acid for 1 minute, root/solution ratio being 1 : 10. In the method using isotopic exchange with Ca^{45}, roots were presaturated with 0.001 M calcium chloride and held for 20 minutes at pH 7.0 by adding calcium hydroxide. Roots were then washed in distilled water, lightly dried, and put on a rotary shaker with neutral 0.001 M calcium chloride containing labelled calcium (5 μc 1000 ml). Root solution ratio was 1 : 20. It was assumed that under these conditions there was no nett transfer of Ca^{++} ions between the roots and the $CaCl_2$ solution. Small samples were drawn from the clear solution at intervals and assayed for Ca^{45} in a scintillation counter. Total amount of exchangeable calcium in roots is given by $\frac{I - F}{F} \times [Ca]$ where [Ca] = concentration in milli equivalent. Fractional exchange (F) is the ratio of the count rate of Ca^{45}-labelled $CaCl_2$ in equilibrium with the roots to the initial count rate of the Ca^{45}-labelled $CaCl_2$ solution. Table 2 shows changes in CEC with time of roots of pea seedlings and of wheat. In

TABLE 2

Effect of time of reaction on CEC values estimated by exchange with Ca^{45}						
	Time (minutes)					
Species	1	5	10	30	60	120
Pea (seedlings)	—	18.5	21.6	23.6	23.0	24.3
Wheat	13.5	15.4	15.1	16.5	18.9	19.0

general only small increases occurred after 10 minutes, but there was occasionally a decrease with time, caused either by other reactions setting in, or by breaking up of root material during the shaking. pH values at the end of 10 minutes were usually between 5.5 and 6.0. Soils can be characterised by the proportion of isotopically exchangeable P to total P, so roots were similarly analysed for total Ca and isotopically exchangeable Ca^{++} after presaturation with non-labelled calcium.

TABLE 3

Isotopically exchangeable Ca per cent of total Ca of calcium-saturated roots		
Plant species	Total calcium me/100 g	Percentage Ca exchangeable against Ca^{45}. Time 2 min
Wheat	10.7	100
Pea seedlings	18.0	98
Cress	41.8	96

The recovery values (Table 3) show that calcium under these conditions is fully exchangeable, and the results preclude the possibility of any chelation.

There are few data on CEC values obtained by different methods. Williams and Coleman [36] usually found similar CEC values using exchange with Ba^{++} or NH_4^+ cations at pH 7.0, whereas values for exchangeable H^+ replaced to pH 8.2 were generally higher.

Table 4, a and b, compares the CEC values of some plant species obtained by various methods and gives mean CEC values of the methods. There was a fair agreement between CEC values obtained by exchange reactions between roots and various cations. Higher values were sometimes obtained by estimation of CEC by neutralising hydrogen-saturated roots. Table 4b shows the reproducibility

TABLE 4

A. Comparison of CEC values by various methods

Plant species	Titration with KOH, pH 2–7	Exchange with		Isotopic exchange with Ca^{45}
		Ba^{++}	K^{+}	
Peas	48.5	45.5	43.0	—
Peas	49.0	42.4	—	44.3
Barley	22.0	17.6	—	15.8
Wheat	14.7	12.6	13.8	—
Wheat	20.7	21.0	17.5	20.9
Clover (red)	53.0	46.1	—	44.3
Clover (red)	—	16.4	15.6	—
Clover (red)	—	24.0	—	22.8
Perennial ryegrass	25.0	23.5	—	18.8
Cress	49.1	51.4	—	—
Sitka pine seedlings	13.9	12.2	—	—
Marrow	—	23.7	—	27.0

B. Mean CEC values estimated by various methods

Method	Species	No of estimations	Mean CEC	S.E. of mean
Titration with KOH pH 2–7	Ryegrass	6	26.4	± 1.21
Exchange with KCl (1.0 *N*)	Cocksfoot	6	16.3	± 1.06
BaCl (0.01 *N*)	Peas	8	43.8	± 0.88
BaCl (0.01 *N*)	Ryegrass	6	22.8	± 0.41
By isotopic exchange using Ca^{45}	Maize	8	11.1	± 0.54

of various methods. Differences in CEC values of fresh and dried root material were on the whole small. Thus CEC values of fresh, air-dried or oven-dried (70°C) perennial ryegrass were 24, 22, and 22 respectively. From the small differences in CEC values between living and dead material, it is obvious that acidic groups originating from metabolic activity were not concerned in the above exchange reactions. Some workers have used root material previously killed by immersion in ether. Williams and Coleman [36] found no objection to this procedure, whereas Helmy and Elgalaby [13] reported progressive lowering of CEC values with time of immersion in ether. In the present work immersion in ether lowered CEC values considerably with increasing time of immersion, but prolonged exposure to ether vapours did not. This latter treatment, unlike immersion, would have no solvent effect. CEC values given in the following account are average values of at least two estimations

by one of the above methods. Exchange with potassium chloride was not used. Within any one set of comparisons the same method was used.

PART II

FACTORS AFFECTING CEC AND RELATIONSHIP BETWEEN CEC VALUES AND K/Ca RATIOS OF PLANT TOPS

CEC values of plants grown in various media

Because most of published CEC values refer to plants grown in pot culture, plants of similar age growing under different and undefined conditions were compared. Table 5 shows results with various species.

TABLE 5

CEC values of roots of plants grown in field or glasshouse

Species	Field samples		Pot-culture samples	
	Sandy loam	Clay with flints	Flint	Sand + vermiculite
Perennial ryegrass .	23	24	23	23
Clover	14	16	46	21
Barley	—	17	23	—
Winter wheat . . .	10	14	18	—

Samples from the field refer to plants grown in a heavy clay with flint (Rothamsted) or on a sandy loam (Woburn) and CEC values quoted are averages of samples from several localities on these soils. Grasses showed the greatest constancy of CEC in various media; other plants from the field usually had lower values. This was particularly evident with red clover, where CEC of field-grown samples were less than half of those of clover grown in flint in the greenhouse. CEC values also differed between clover grown in flint or in a mixture of sand with vermiculite. Although some finer roots might be lost when sampling field plants, no such losses should have affected comparison between the two greenhouse samples. According to Jacobson and Overstreet [19], it is doubtful whether root hairs contribute substantially to exchange adsorption. The results could thus indicate a certain adaptibility of some plants to different media.

CEC and age of plant

Most studies on effect of age on cation-exchange capacity have been restricted to plants less than 1 to 2 weeks old. Changes in CEC

of very young plants may reflect rearrangements in the chemical composition of the cell walls of roots, but their effect on nutrient uptake is unlikely to be great. After this early stage such changes may be important in the adsorption processes of nutrients. Winter wheat growing in the field and sampled monthly between one to seven months showed small and not consistent changes in CEC. Other plants varied, *e.g.* pea plants gave increased CEC values between seedling and flowering stage, while CEC of a number of plants, *i.e.* maize, kale decreased with age. Several months-old kale exhibited considerable variations within one root system, depending on whether finer or coarser root parts were estimated. CEC of clover decreased with age. Thus two- and six-months-old clover plants grown in the field had CEC values 16 and 8 respectively.

Effects of nutrient levels on CEC

Several workers [16 33] have shown that the level and type of nitrogen affect CEC of young roots. McLean *et al.* [24] found a high correlation between total nitrogen content of roots and their CEC values. In the present work, CEC of 4-months-old perennial ryegrass was increased from 21 to 26 by applying 100 pounds N per acre as ammonium sulphate to a N-deficient soil in the greenhouse, and the same amount of N applied as ammonium sulphate, calcium nitrate, or urea increased CEC of Italian ryegrass grown in the field from 25 to 32, 34, and 26 respectively. On an old established mixed sward, a similar amount of ammonium sulphate had no effect 2 months later on CEC of grass roots. Wheat (2 to 6 months old) growing in the field showed only small differences in CEC values for nitrogen applied as ammonium sulphate or calcium nitrate on a soil where wheat showed no response to nitrogen. No close relationship was found between CEC of various species and the nitrogen content of their roots, which agrees with results by Blanc-Aicard [2] for various grasses. This lack of relationship does not support the suggestion [32] that cation-exchange sites of roots are of a nitrogenous nature, but is consistent with the view [2] that nitrogen additions stimulate root growth. Phosphate added to a P-deficient soil increased significantly CEC of perennial ryegrass. These changes of CEC values generally agree with findings by other workers. Table 6 examines the compatibility of results obtained by

various workers using different methods with living or dead root material of diverse age and grown under undefined conditions.

TABLE 6

Comparison of CEC values estimated by various workers

Authors	Species						
	Wheat	Barley	Maize	Buck-wheat	Red clover	Peas	Perennial ryegrass
Drake *et al.* [6]	9	12	26	—	47	—	24
McLean *et al.* [24] . . .	14	13	13	39	—	—	24
Mattson [25]	—	23	—	—	—	71	—
Graham and Baker [11]	20	23	—	—	—	—	—
Blanc-Aicard [1] . . .	—	—	25	—	—	44	38
Keller and Deuel [21] .	23	—	29	—	—	—	—
Heintze.	18	22	14	38	46 *	48	23

*) Grown in flint

The differences between results of various workers are generally not greater than those found when a species grown under different conditions; the compatibility of results suggests that CEC reflects a characteristic constituent of the plant root not connected with metabolic processes.

CEC values and uptake of potassium and calcium by plants

Mattson [25], Mattson and Larsson [26], Elgalaby and Wiklander [8 9] studied the relationship between root cation-exchange capacity and cation uptake by plants and showed that, under certain nutrient conditions, plants with high CEC favoured divalent to monovalent cation uptake and *vice versa*. In an attempt to evaluate the effect of CEC on cation uptake, Wallace and co-workers [30 16] found that the following equation satisfied most of their results:

$$\frac{CEC_1}{CEC_2} = \frac{K_2}{K_1}$$

where 1 and 2 denote two plant species or one species at two nutrient levels, and K_1 and K_2 the corresponding potassium contents of plant tops. Table 7 gives CEC determinations and analyses of tops of plants grown in flint or sand-vermiculite mixture. In the flint series, plants were grown at two K-levels, "high" K level corresponding to $^1/_2$K of Hoagland solution, "low" K to $^1/_8$K of

Hoagland, other nutrients being equal to those of $^1/_2$ Hoagland. P- and N-concentrations at low K-level were maintained by substituting some of the potassium in potassium phosphate with sodium and by increasing the calcium nitrate. Dilute Hoagland solution ($^1/_5$) was used for the sand-vermiculite mixture and micronutrient solutions were added to both series. Similar results were obtained on several occasions during the three months growth; results in Table 7 are for samples taken two months after germination.

TABLE 7

CEC values of roots and K/Ca ratios of plant tops of crops grown in flint or in sand + vermiculite at two K-levels. Plants about 2 months old

Exp.	Year	Medium	K-level (expressed as fraction of K-level in Hoagland solution	
			High	Low
I	1958	Flint	$^1/_2$	$^1/_8$
II	1959	Flint	$^1/_2$	$^1/_8$
III	1959	Sand + vermiculite	—	$^1/_5$

Species	CEC values		K/Ca (expressed as me) of plant tops	
	High K	Low K	High K	Low K
Exp. I				
Barley (Herta)	23.0	14.5	1.88	0.61
Barley (Plumage Archer)	18.0	14.0	1.65	0.56
Wheat	16.6	—	2.20	—
Exp. II				
Kale	40.0	31.2	0.54	0.18
Red clover	45.1	39.0	0.73	0.15
Perennial ryegrass	24.0	21.0	1.66	0.66
Cabbage	—	35.0	—	0.24
Red beetroot	—	31.5	—	0.21
Exp. III				
Marrow		41.0	—	0.56
Buckwheat		40.0	—	0.84
Perennial ryegrass	—	22.0	—	2.10
Fescue grass		16.1		3.10
Red clover	—	21.0	—	0.81
Lucerne		13.5	—	0.99
Maize	—	14.3	—	2.10

Lowering the K-level, while simultaneously increasing the Ca-level of the nutrient solution, decreased CEC values. Fig. 2 shows that CEC values grouped within a K-level were related to K/Ca ratios of plant tops in the way found by Mattson *et al.* [27] and

Fig. 2. Relationship between CEC values of roots and K/Ca ratios of tops. Plants grown in flint: — ⊙ = $\frac{1}{2}$K of Hoagland, × = $\frac{1}{8}$K of Hoagland. Plants grown in sand/vermiculite: — ● = $\frac{1}{5}$K of Hoagland.

others [8 9 16 31 32]. In the sand-vermiculite series, however, red clover and lucerne had low CEC values similar to those of grasses, but much lower K/Ca ratios. Clover thus exhibited considerable variations in cation exchange capacity when grown in flint or in sandvermiculite mixtures but maintained similar K/Ca ratios. The distribution of points suggests that there is a wider variation in K/Ca ratios for plants with CEC values ranging from 20 downwards, than for those with higher cation exchange capacities. A few roots as well as plant tops were analysed for potassium and calcium

content. Results (Table 8) show that at both K-levels in the flint series clover roots had higher K/Ca ratios than grass roots, whereas the reverse was found for tops and the whole plant. In the plants grown at high level of K, the contents of both K and Ca (expressed as per cent of dry matter) of whole plants were higher in clover than in grass; at the lower K-level, where the Ca-level was simultaneously increased, the K-content of both clover and grass was lower than at high K-level, but the Ca-level was unaltered in clover and increased in grass.

TABLE 8

Comparison between CEC of roots and K/Ca ratios of roots and plant tops

Plant species	K-level	CEC	K/Ca (expressed as me)	
			Plant tops	Roots
Red clover	High	45.1	0.73	3.24
	Low	39.0	0.17	0.90
Perennial ryegrass . .	High	24.0	1.66	0.94
	Low	21.0	0.66	0.48
Maize	~ High	14.3	2.1	0.28

In a comparison of 24 pairs of plant species grown under similar glasshouse conditions, the correlation coefficient between CEC values of roots and the inverse potassium ratios of the plant tops was just significant ($r = + 0.47$). Huffaker and Wallace [17] also found a significant correlation coefficient (+ 0.88) for this relationship for 3 crops at 4 levels of nitrogen, but they stressed that the relationship seemed far from simple and did not hold in several cases. Smith and Wallace [30] advanced several causes for low correlation coefficients being obtained, such as the level of nutrients allowing luxury uptake, and the insufficient accuracy of CEC estimations for the ratio treatment.

DISCUSSION

The titration curves of roots failed to elucidate the nature of the acidic groups on root surfaces, but they give independent evidence that pectin is involved in cation-exchange capacity, a theory first advanced by Devaux [5] in 1916 and recently again propounded by Mattson *et al.* [27] and Keller and Deuel [21]. The rapid acid drift when acid root suspensions undergo neutralisation, was similar in nature to that found by Holden [14] with minced leaf extracts. In

experiments comparing several methods for estimating CEC of roots, exchange reactions with K^+-ions were more sensitive to factors such as concentration of exchange solution, the duration of its contact, and that of the subsequent rinse in water, than were those with Ba^{++}. At concentrations below normality roots greatly preferred Ba^{++} ions as against K^+-ions. Similar differences between the adsorption of mono- and divalent ions have been observed by other workers [9 10], and Blanc-Aicard [1] proposed that exchange capacity for specific cations, *e.g.* calcium, should be substituted for the concept of a general cation exchange capacity of roots. For exchange reactions a brief contact, not exceeding 30 seconds, between root and solution was recommended by Williams and Coleman [36], while Hope [15], who regards the first step in cation uptake to be a reversible diffusion process, considered that active accumulation follows after 10 to 20 minutes of diffusion. In these experiments with roots presaturated with calcium for 20 minutes, all the adsorbed calcium was subsequently exchangeable against Ca^{45}. Small differences between the CEC values of living and air-dried root material confirmed that exchange-capacity measurements do not involve metabolic activity of the root. Values for clover species quoted in the literature fall between 40 and 50; for red clover similar values were obtained in this investigation from plants grown in flint, but values from 5 to 22 were found from plants in the field or raised in a sand—vermiculite mixture. Smith and Wallace [32] obtained lower CEC values for citrus seedlings grown in the field than under glass, and Wiersum and Bakema [34] observed a CEC adaptability to the growth medium when comparing roots grown in resins supplying either cations or anions only. In the present work evidence was obtained that exchange capacity was connected with the relative adsorption of mono- and divalent cations, but considerable differences were found in the K/Ca ratios of some plants possessing similar CEC values. This, together with considerable variation in cation-exchange capacity within one species, while its K/Ca ratio changed very little, suggests the importance of other factors besides CEC in the train of events leading to the final absorption of cations by plants. Conclusions reached under glass may not apply to the field; this is illustrated by similar CEC values obtained for clover and kale grown in flint, although in potassium-deficient field soils kale, but not clover, can

establish itself. In a recent study of competition between white clover and some grass species, Mouat and Walker [29] postulated the importance of rates of emergence, because CEC values alone were too similar to explain some results. Until more is known of the active absorption from metabolic activity and of its effect on gross responses, it is better not to over-emphasise the importance of CEC values in the mineral nutrition of plants.

SUMMARY

1. Enzymic breakdown of pectin caused an initial rapid pH drift towards the acid side when hydrogen ion-saturated roots were neutralised.

2. Buffer curves of root suspensions showed that at least some of the acidic groups of root surfaces are derived from carboxylic groups of pectic acid.

3. Roots showed greater affinity for Ba^{++} than for K^{+}-ions at concentrations below normality. Estimations of CEC values from exchange reactions between roots and the latter cations were more affected by altering the conditions during exchange than were those from exchange with Ba^{++}.

Isotopic exchange between roots saturated with non-labelled calcium and Ca^{45} suggested that adsorbed calcium is held in an exchangeable and not in chelated form.

4. Changes in CEC caused by aging of plants, or by varying nutrient levels in the growing medium, agreed in general, both in direction and magnitude, with the findings of other workers.

5. High CEC values were associated with low K/Ca ratios of plant tops or of the whole plant, and *vice versa*, when comparisons were restricted within the same K-level of the growing medium. This relationship was reversed for CEC values and K/Ca ratios of roots of clover and ryegrass.

6. A correlation coefficient that was just significant was found in a number of comparisons for the relationship between CEC values of two species and the inverse ratio of the K-contents of their plant tops.

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FUNGI ASSOCIATED WITH THE SEEDLING ROOTS OF *ALLIUM PORRUM* L.

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INTRODUCTION

Information is gradually being accumulated (Harley and Waid [2]; Peterson [3]; Stenton [4]) on the nature of the saprophytic mycoflora intimately associated with roots (*i.e.* the root surface and root tissue mycofloras), but there is still the need for more qualitative information on the development of fungi on the root surface and within roots (Peterson [3]). The conditions which affect the development of fungi on and in roots are not clearly understood; the effect of light intensity on the fungus flora of beech roots (Harley and Waid [2]) and the effects of plant age and soil type (Peterson [3]) are isolated examples of detailed studies on factors influencing the nature of the root mycoflora.

The aim of this investigation is to provide further information on the effect of soil type on the development of fungi associated with roots. In view of the interesting results provided by Timonin and Thexton [5] on the microfloras of the root region of onion and garlic (*Allium cepa* and *A. sativum*), which suggest very sparse microbial colonization of the roots, the roots studied here are those of the related species, leek (*A. porrum*).

MATERIALS AND METHODS

Leek seedlings (variety Musselburgh) were grown under greenhouse conditions in three soils. These soils (Broadbrook, Woodhead, and Experimental Plot) were cultivated soils collected from Wirral, Cheshire, and brief data on these soils are given in Table 1. Table 2 indicates the fungus population of the three soils, this information being obtained by the soil plate technique (Warcup [6]).

TABLE 1

Soil	Woodhead	Broadbook	Experimental Plot
pH	5.6–6.0	7.6–7.8	5.1–5.5
Percentage loss on ignition	8.4	11.9	12.1

TABLE 2

Fungi isolated from the three soils under experiment by the Warcup soil plate method (Results expressed as percentage frequency of occurrence)

Soil	Broadbook	Woodhead	Experimental Plot
Fungi isolated			
Absidia sp.	4	4	4
Cunninghamella sp.	4	—	—
Mortierella spp.	36	16	20
Mucor ramannianus	—	—	66
Mucor sp.	4	2	4
Pythium sp.	2	—	—
Zygorrhynchus moelleri	2	6	18
Chaetomium sp.	—	16	8
Gelasinospora cerealis	4	—	2
Alternaria sp.	2	—	—
Aspergillus spp.	—	6	—
Cephalosporium sp.	—	2	—
Fusarium culmorum	2	2	2
Fusarium spp.	2	2	4
Gliocladium sp.	—	2	—
Oospora sp.	4	30	2
Penicillium spp.	30	36	36
Pullularia pullulans	12	—	—
Sphaeropsidaceae	12	6	12
Stysanus sp.	—	4	—
Torula sp.	4	—	2
Trichoderma viride	34	44	70
Sterile dark mycelium	14	2	2
Sterile white mycelium	2	2	—
Sterile yellow mycelium	8	2	—

The seedlings were sampled at 30 to 35 days after sowing. In the laboratory the roots were washed first with tap water, until all visible soil particles had been removed, and then with numerous changes of sterile water (Harley and Waid [2]). The washing technique used was to place samples of 5 roots, after tap-water washing, in vials containing 10 ml sterile water, and to wash each root sample with 10 changes of sterile water (transferring the roots to a fresh sterile vial after the 4th, 8th and 10th washings). Preliminary tests had shown that 10 washings with sterile water allowed efficient washing of leek-seedling roots.

After washing, as much surface moisture as possible was removed from the

roots, using sterile filter paper, and then the roots were cut into segments (length 2 mm). These segments were placed in Czapek-Dox agar supplemented with yeast extract (pH 5.0) in petri dishes (5 segments per dish). The dishes were incubated for 3 days at 25°C and thereafter kept at room temperature. Fungi developing from the root segments were isolated into pure culture and identified.

Root samples were also taken for investigation using direct microscopic observation. These roots were washed in the manner previously described, after which they were stored in formalin—acetic—alcohol. Prior to direct observation the roots were transferred to lacto-phenol for several hours, then stained in 0.5 per cent cotton blue in lacto-phenol (warm) for 2 minutes. After this excess stain was removed in lacto-phenol, and the stained roots were cut into 2-mm segments and mounted in lacto-phenol. Each segment was then examined microscopically for the presence of hyphae and, where present, the type of hyphae was recorded.

RESULTS

Details of the fungi isolated from primary roots of leek seedlings grown in the three soils under experiment are given in Table 3. The percentage-frequency figures given being based on samples of 15 to 25 roots (*i.e.* 318 to 338 plated root segments). The data ob-

TABLE 3

Fungi isolated from the washed roots of 30 to 35-day-old leek seedlings grown in the three soils under experiment (Results expressed as percentage frequency of occurrence)

Soil	Broadbrook	Woodhead	Experimental Plot
Fungi isolated			
Cylindrocarpon spp.	25.4	16.3	11.8
Fusarium spp.	0.6	3.4	13.9
Gliocladium spp.	—	0.3	3.6
Helminthosporium sp.	—	0.3	—
Mortierella spp.	—	0.6	4.1
Mucor ramannianus	—	—	2.4
Penicillium spp.	0.6	0.6	3.3
Periconia sp.	—	—	1.2
Pullularia pullulans	0.3	—	—
Sphaeropsidales	—	0.3	1.2
Sterile hyaline forms	6.6	4.4	1.2
Sterile dark forms	4.7	29.6	8.3
Trichoderma viride	—	—	0.6
Unidentified spp.	0.9	0.6	0.9
No. of segments plated	318	318	338
Per cent segments colonized by fungi	36.5	55.9	48.8

tained from the direct observation of the roots is given in Table 4 (here the percentage frequency figures are based on observations of

TABLE 4

Results of direct observation of leek seedling roots grown in the three soils under experiment			
Soil	Broadbrook	Woodhead	Experimental Plot
Percentage root segments showing hyphae	57.8	45.7	55.8
Percentage root segments showing hyaline hyphae	55.5	32.1	55.0
Percentage root segments showing dark hyphae	7.5	17.9	2.9
Percentage root segments showing both hyaline and dark hyphae	5.2	4.3	2.1

root segments from 20 to 25 roots. From this data several generalisations may be made:

1. It can be seen, comparing Tables 2 and 3 that the fungi most frequently associated with leek roots are not isolated with high frequency, by the soil plate method, from the soil distant from the roots.
2. The roots are by no means completely colonized by fungi (the percentage of segments yielding fungi in the cultural method varying from 36.5 to 55.9, and by direct observation of root segments this variation is between 45.7 and 59.1 per cent).
3. Direct observation of roots indicated hyaline hyphae to be most frequently associated with the roots, but on roots from the Woodhead soil dark hyphae were observed with much greater frequency than with roots from the other two soils.
4. For plants grown in the more acid soil (Experimental Plot) there is less Cylindrocarpon and more Fusarium associated with the roots than with the roots of plants grown in the alkaline soil (Broadbrook).
5. Species of Gliocladium and Mortierella are mainly associated with the roots of plants grown in the acid (Experimental Plot) soil, although they occur with a very low percentage frequency on roots from the Woodhead soil which is also slightly acid.
6. Only in plants grown in the Woodhead soil is the mycoflora associated with the roots dominated by sterile forms (in particular sterile, dark forms).

DISCUSSION

The data provided in Tables 3 and 4 show that fungal colonization of the seedling roots of leek (*Allium porrum*) is not complete; the percentage of root segments colonized, as indicated by the isolation method, varying from 36.5 to 55.9, or, as indicated by direct observation, varying from 45.7 to 57.8. In comparison, roots of broad-been seedlings of similar age show a much greater degree of fungal colonization (70 per cent of plated root segments yielding fungi).

Timonin and Thexton [5] in their work with other species of Allium (*A. cepa* and *A. sativum*), using the dilution plate method to assess numbers of fungi in the rhizosphere and on the root surface, showed very low numbers of fungi present on the root surface of these species. Thus their results suggest an extremely small amount of fungal colonization of the roots. The data provided here, for another species of Allium (*A. porrum*), supports the idea that the degree of root colonization is not so great as for the roots of many Angiosperms, yet it is not so sparse as to provide data of the type given by Timonin and Thexton [5].

It has been suggested (Clarke and Parkinson [1]) that the maceration of the root tissues, which forms part of the technique used by Timonin and Thexton, when applied to roots of Allium species, causes the release of substances which inhibit fungal growth. Allium roots which have not been subjected to maceration yield fungi in greater numbers than do macerated roots. This may explain the low numbers of fungi recorded from the roots of *A. cepa* and *A. sativum* by Timonin and Thexton [5].

The species isolated from the roots of *Allium porrum* (Table 3) are, for the most part, species which have come to be regarded as typical root surface or root tissue fungi. *Cylindrocarpon* spp., *Fusarium* spp. and sterile forms dominated the isolates, with *Gliocladium* spp., *Mortierella* spp. and *Penicillium* spp. isolated less frequently. Few of the fungi associated with the roots are isolated with any degree of frequency from the soil distant from the roots; *Mortierella* spp. and *Penicillium* spp. are the only root forms which were isolated from the soil with high frequency (Table 2). It seems that many of the above-mentioned genera find a suitable ecological niche on or in living roots. Much more aut-ecological information

is required on the growth and survival of these species in the soil, and on their capacities as saprophytic root colonizers.

The data provided by direct observation of the roots is an essential complement to isolation studies. Comparing the data provided in Tables 3 and 4 on the colonization of roots by fungi, *i.e.* data provided by isolation and by direct observation, it can be seen that for roots grown in each soil divergent results were given. For roots grown in the Broadbrook and Experimental Plot soils direct observation showed greater fungal colonization than did isolation, but roots from the Woodhead soil showed the opposite effect.

In observing the roots it was noticeable that the root hairs were very rarely colonized, superficially or internally, by fungal hyphae. Where hyphae were present with high frequency their growth appeared to be mainly parallel to the longitudinal axis of the root; between these hyphae cross connexions were often visible. Although chlamydospores were occasionally seen, conidiophores or conidia were never observed in this investigation, and the majority of the sterile hyphae observed were hyaline forms. For roots grown in the Woodhead soil much greater colonization by dark hyphae was observed than for roots grown in the other two soils. This data is paralleled by the isolation data (Table 3), where a relatively high percentage occurrence of sterile, dark mycelium was recorded from roots grown in the Woodhead soil. It is tempting to correlate these findings from the two methods of study, and to infer that the dark mycelium observed on the roots represents the dark sterile forms isolated from the roots.

The effect of soil type on the development of populations of fungi closely associated with roots of red clover and wheat has been discussed by Peterson [3]. He noted striking differences between root surface fungi of roots from acid and alkaline soils, particularly with respect to the occurrence of *Fusarium* spp. and *Cylindrocarpon* spp. (*Fusarium* spp. being predominant on roots from acid soil, *Cylindrocarpon* spp. predominant on the roots from alkaline soil).

The results provided here substantiate this observation, although on roots grown in the acid Experimental Plot soil *Fusarium* spp. are not predominant, but share dominance with *Cylindrocarpon* spp. The results provided in Table 3 also show the occurrence of *Mortierella* spp. and *Gliocladium* spp. to be on roots grown on acid

soils; this is different from the situation demonstrated by Peterson [3] for roots of red clover and wheat, where these genera were isolated most frequently from roots grown in non-acid soils.

It would seem that the explanation of these phenomena lies in the varying sensitivity of the individual root surface fungi to soil reaction (Peterson [3]), but at the same time it must be emphasized that more details on the biology of the individual species are required to allow real understanding of the development of populations of saprophytic fungi in close association with plant roots.

SUMMARY

Data is provided on the fungi isolated from the roots of *Allium porrum* which have been grown in three soils. These data show that the roots support a typical root-surface mycoflora, although this is more sparse than that recorded for several other Angiosperm roots.

The results indicate that, as suggested by Peterson [3], soil pH influences the association of saprophytic fungi with plant roots. Species of Cylindrocarpon, Fusarium, Mortierella and Gliocladium are fungi which seem to be most affected in this respect.

The need for detailed studies on the biology of individual species, which frequently occur in close association with plant roots, is emphasized.

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THE SITES OF EXCRETION OF NINHYDRIN-POSITIVE SUBSTANCES BY BROAD BEAN SEEDLINGS

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INTRODUCTION

The stimulating influence of root exudates on soil micro-organisms is regarded as one of the main causes of the rhizosphere effect. Of these exudates, amino acids are considered to be an important fraction. Although data are available on the effect of environmental factors on amino acid excretion from plant roots (Katznelson, Rouatt, and Payne [1]; Rovira [3]), and on differences in exudates from different Angiosperms (Rovira [3]), there is little information on the sites of such excretion on a root.

Knowledge of the sites of excretion of organic materials may allow greater understanding of the speed and nature of the development of rhizosphere and root-surface microfloras of very young seedlings. This investigation attempts to locate the sites of excretion of ninhydrin-positive substances from germinating seed and from the emerging roots of broad bean. Although it is probable that amino acids form the bulk of the material herein referred to as "*ninhydrin-positive substances*", in the absence of detailed characterization it is not possible to use other than the general term (a positive colour reaction with ninhydrin is given by a range of substances, including organic amines, polypeptides and ammonia).

METHODS

The seeds and roots studied were of broad bean (*Vicia faba*), variety "Conqueror". Before experimentation the seeds were surface sterilised by immersing in Nance's solution for 10 minutes (preliminary tests had shown

that this gave effective surface sterilisation without imparing the germinating capacity of the seed). The seeds were then thoroughly washed in sterile water to remove all adhering, sterilising fluid.

After surface sterilisation, 10 seeds were removed for immediate investigation, while the others were planted in pots and kept in a greenhouse (minimum temperature 55°C). Each day, up to 7 days after planting, a sample of 10 seeds was taken, and these tested for the exudation of substances giving a positive colour reaction with ninhydrin. Germination occurred 3 to 4 days after planting, the oldest roots were therefore about 3 days old, and showed no development of root nodules.

The selected beans, handled gently with forceps to avoid contamination, were carefully wrinsed in tap water to remove as much soil as possible, without injuring the tissue in any way, and after washing were placed on filter paper inside sterile, glass-covered enamel dishes. Whatman No 50 proved to be the most suitable absorbent paper. A second sheet of filter paper was laid on top of the beans, and moist sheets of Kleenex tissue placed on top and around this, so that the upper sheet of filter paper folded round the bean, and the whole system was kept moist.

The dishes were kept at 25°C for 24 hours. After this time the beans were carefully uncovered and the position of each bean seed, and root if present, on its piece of filter paper was marked by piercing small holes in the filter paper beneath the seed with a flamed needle. The beans were then removed and placed in separate labelled petri dishes which contained moist filter paper. The beans were kept in this moist environment for sufficient time to allow careful inspection of the root for damage where anomalous exudation patterns had been obtained.

The filter papers which had been placed beneath the beans to absorb any excreted substances were dried in a gentle current of warm air, then sprayed with 0.25% ninhydrin in ethanol, and dried again. The outline of each bean was then drawn on the filter paper using the guide holes in the paper, and the papers placed in a dry cupboard at room temperature for two days, after which time the ninhydrin coloration patterns showed up clearly.

Even though care had been taken in removing the seeds and roots from the soil, it was still possible that the release of "ninhydrin-positive substances" was due to the damaging of root hairs on removing the roots from the soil. Other possible reasons for the detection of ninhydrin-positive substances are that the surface-sterilising solution could have damaged the cells of the seed coat, or that the ninhydrin-positive substances recorded were released, not by the root, but by micro-organisms, either directly or as a result of their action on root and seed coat cells. These possibilities were investigated as follows.

To investigate the possibility that the chemicals of the surface-sterilising solution damaged the cells of the seed coat in any way, thus releasing ninhydrin-positive substances, samples of unsterilised beans were used in the manner previously described. Other samples of surface-sterilised beans were grown in sterile soil and supplied with sterile water. Ninhydrin colour patterns obtained under these conditions, when no micro-organisms were

present, were caused by substances released from the higher plant tissues.

If the ninhydrin-positive substances came from damaged root hairs, then the maximum exudation would be expected to occur in that region of the root clothed in root hairs at the time the root was removed from the soil. Some roots, after washing, were then examined under a binocular microscope and the position of the root-hair zone, in relation to the root tip, recorded. When these roots were placed on filter paper the positions of the seed and the root tip were marked. After 24 hours the new positions of the root tip and root-hair zone were determined. A full-scale drawing (Fig. 3) was made showing the relative positions of the original root-hair region and root tip, the new root-hair region and root tip, and the coloration pattern produced after spraying the filter paper with ninhydrin solution.

RESULTS

The ninhydrin coloration patterns obtained from ungerminated seeds, and from germinated seeds and their young roots, can be seen in Figures 1 and 2. These figures show the patterns obtained

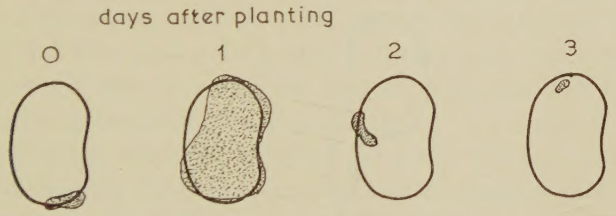

Fig. 1. Ninhydrin colour patterns obtained from germinated seeds ($^2/_3 \times$ orig. size).

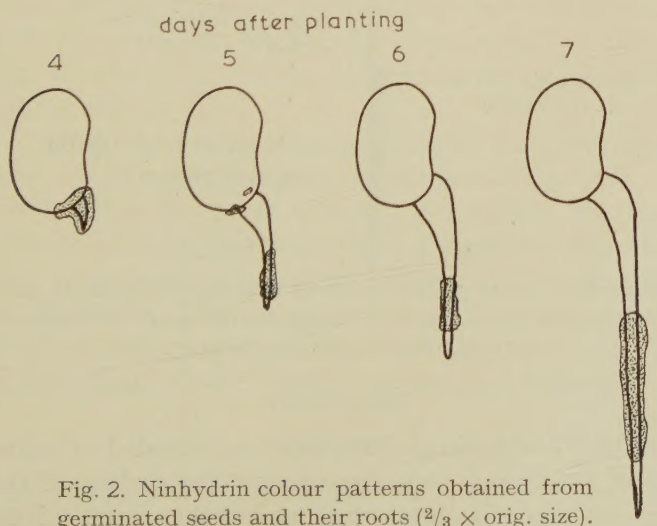

Fig. 2. Ninhydrin colour patterns obtained from germinated seeds and their roots ($^2/_3 \times$ orig. size).

when surface-sterilised seeds were planted in unsterilised soil. Controls, using seeds, which had not been surface sterilised, planted in unsterilised soil, and surface-sterilised seeds planted in sterile soil, showed similar patterns. The controls showed that the surface-sterilising solution had no effect upon the excretion of ninhydrin-positive substances from the root, and that the ninhydrin-positive substances detected came from the seed and root tissue and were not caused by the presence of micro-organisms on the root surface.

The ninhydrin coloration patterns obtained from the seed coats were somewhat variable, but did show that in general excretion began in the micropylar region. In contrast the excretion patterns obtained from the roots were constant within quite narrow limits.

Figure 3 shows that the presence of ninhydrin-positive substances is not caused by the damaging of the root hairs on removing the root from the soil.

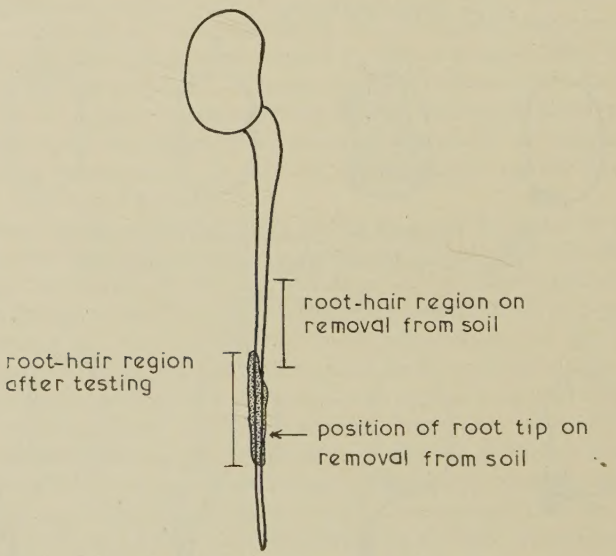

Fig. 3. Ninhydrin colour pattern showing that the detection of ninhydrin-positive substances is not due to damage of root hairs on removal of the roots from soil ($^2/_3$ × orig. size).

DISCUSSION

Excretion of ninhydrin-positive substances tended to begin in the micropylar region and then spread over the surface of the seed reaching a peak one day after planting, but then quickly falling off.

With the emergence of the new root, ninhydrin-positive substances were detected around the emerging root. At first it seemed that the whole surface of the root was excreting ninhydrin-positive substances, but as the root grew, it became obvious that a limited region of the root, behind the root tip, was responsible.

In some cases anomalous excretion patterns were obtained. On examining the roots concerned, in the majority of cases these anomalous patterns were found to be due to some slight damage to the root. In some cases, anomalous patterns were found to be due to the presence of fungal hyphae, but on microscopic examination of the filter paper, any coloration caused by the reaction of ninhydrin on the hyphae was easily distinguished from that caused by the true exudates.

Robinson [2] cut sections of the root of the broad bean (variety Aquadulce), and analysed them for amino-nitrogen content (as a measure of proteolytic activity). She showed that the level of amino nitrogen had two peaks. In a root 140 mm long, the peaks were 30 mm and 100 mm from the tip. In this study some of the roots, 4 days after germination, reached lengths up to about 100 mm. Special attention was paid to such long roots. Taking the centre of the region showing the excretion of ninhydrin-positive substances as being the point of maximum excretion, this was found to lie about 28 mm behind the tip. This point corresponds to the first peak of proteolytic activity found by Robinson. The second peak corresponds to the region of eruption of lateral roots. In this study, excretion of ninhydrin-positive substances by lateral roots was observed but not recorded.

There is little doubt that microquantities of ninhydrin-positive substances may be released from any region of the root surface, particularly when the root is wounded in any way. However, this study shows that in healthy roots there is a region, behind the root tip, which is particularly important in the excretion of ninhydrin-positive substances. This region is limited in extent, and the excretion of ninhydrin-positive substances is correlated with the high level of proteolytic activity inside this portion of the root.

SUMMARY

A simple method is described for determining the sites of excretion of ninhydrin-positive substances from the undamaged roots of seedlings of

broad bean. Results are given on the sites of excretion of such substances from the germinating seed and from the young root. It is suggested that in such roots the centres of maximum excretion may be correlated with centres of high proteolytic activity within the root.

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